

Towards colorimetric anion-selective indicators:
Solid-phase methods for azo dye synthesis and screening

Research Thesis

Presented in partial fulfillment of the requirements for graduation “with honors research distinction” in the undergraduates colleges of The Ohio State University

By

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Abstract

Development of an aqueous colorimetric anion indicator is imperative to the health and well-being of the peoples of remote areas where arsenate, cyanide and sulfide contaminate much of the drinking water. Very few examples exist that retain their anion selectivity in water, so many molecules must be synthesized and analyzed to determine which functional motifs warrant the development of an exhaustive structure-activity relationship for aqueous colorimetric anion indication. Sudan Black B, a commercially available dye, which possesses a crude colorimetric effect in the presence of bromide, inspired the synthesis of similar molecules. A planar support was developed to allow for the time-efficient, parallel synthesis of an array of azo compounds, which could be analyzed, without cleavage, by UV-Vis-NIR spectroscopy for convenient determination of their structure and colorimetric effect.

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Chapter 1: Motivation and previous work

1.1 The necessity of an aqueous colorimetric anion indicator

Anions play a critical role in innumerable biological, environmental and industrial processes. They are directly involved in the mechanisms of many diseases, are important resources for most living species and are crucial to the production and degradation of many industrial commodities. Detection of these anions can provide an understanding of the effects of these anions on the multitude of processes in which they are involved. Although a wide variety of techniques for detecting anions exists, an instrument-free method would provide a superior means to quantify concentrations in field applications where instrumentation is impractical.^{1, 2, 3, 4}

The determination of pollutants in the water supply is one of the most important applications of anion detection, especially since anions like cyanide and the oxides of arsenic (AsO_3^{2-} , AsO_4^{3-}) are severely detrimental to health. Cyanide disrupts cellular respiration through inhibition of the cytochrome c oxidase and arsenite/arsenate disrupts the synthesis of ATP in the cell through the Krebs cycle and the electron transport chain, which through acute or chronic toxicity results in death.⁵ These contaminants are especially worrisome in many Asian regions like Bangladesh and West Bengal, where a staggering 72% of districts had arsenic levels above the WHO acceptable maximum.⁶ Within these regions, there are more than 145 million people affected by this contamination. Since technical and financial barriers prevent the availability of analytical instrumentation in impoverished areas of the world, an efficient method to detect these contaminants that does not require extensive training or instrumentation would be extremely valuable.

There are several methods currently available for the detection of anions, two of which are instrumental approaches and another two that rely on molecular interactions. Ion chromatography (IC) and ion selective electrodes (ISE) use instrumentation to detect anions. IC uses a high-performance liquid chromatography column to separate ions based on columbic interactions and then detects the eluent by UV-vis spectroscopy or conductivity to determine the identity of each eluted ion. This method is excellent for precisely determining the composition of a solution; however, each instrument can cost at least \$50,000, requires intensive training and takes considerable analysis time per sample. Additionally, this instrument is far from portable, which renders it a poor choice for analyzing the contamination of water in the field.⁴

ISEs use the electrochemistry of the anions to measure their presence, yet they are not without limitations. While they show selectivity towards specific anions, they often experience interference from other anions. The most often observed example of this limitation is seen with a fluoride ISE that still responds to hydroxide at one tenth the response level of fluoride. Additionally, ISEs are fragile and require frequent calibration in order to properly determine the concentration of a single anion in a sample.⁷ Although the power and utility of these techniques are inarguable in a conventional laboratory setting, alternative methods hold great value to provide anion concentration results outside the boundaries of the chemistry laboratory.

Instrument-free methods of anion detection are limited because of the unique challenges they present. Anions have a much lower charge-to-radius ratio than cations and thus interactions involving their electrostatic effects must be highly selective. Additionally, many anions are sensitive to pH and can become protonated at low pHs causing a loss of

negative charge, which impedes many potential electrostatic interactions.⁸ On the molecular side of detection, there are two major options: fluorimetry and colorimetry. Both these techniques require an observable change when a molecule of interest (anions) interacts with a chosen indicator. These detectable changes occur when the interaction between an indicator and another molecule affects the way the indicator absorbs, reflects or emits light. Typically, the emitted light has lower energy than the absorbed radiation and is therefore at a longer wavelength, an attribute labeled the Stokes shift. Often, this difference corresponds to energy loss arising from non-radiative decay to the lowest vibrational energy level of the excited state. The wavelength of the light absorbed and emitted are not the same because of the energy lost in the Stokes shift. Molecules that express fluorescence, known as fluorophores, have been widely used throughout biology, chemistry and environmental science as probes to determine the location of other molecules to which they can be attached. While fluorophores cannot be easily attached to anions in a sample, it is possible for the anions to interact with the fluorophores and change their fluorescence.

Fluorophores can be used as indicators through binding interactions that alter the extended π -bonding of the indicator and cause a change in fluorescence or through a quenching effect that prevents fluorescence from occurring. Fluorescent indicators with binding interactions have been used to identify reactive anions such as cyanide through a modified coumarin–spiropyran conjugate (Figure 1).⁹ Due to the large variation in size, geometry and reactivity of anions, binding interactions cannot easily be modified to be selective for different anions. Quenching is the result of another molecule (anions in this case) being able to dissipate the high vibrational energy of the excited electrons in the

fluorophores without the release of light. Frequently, many anions will cause a quenching effect in an indicator, though one anion will be dominant in this interaction. Biosensors have shown significant developments in this area, particularly in the detection of chloride ions due to its importance in neuron function and cystic fibrosis.¹⁰ The fluorescence of yellow fluorescent protein (YFP) can be quenched by several anions including chloride. While common variants of YFP are not very sensitive to chloride, modifications have been made to increase this sensitivity; furthermore, an indicator, Clomeleon, has been developed by linking YFP to cyan fluorescent protein (CFP) through a short peptide chain for ratiometric determination of chloride concentrations *in vivo*.¹¹ This method experiences minimal interference from biologically relevant anions such as carbonate, nitrate, and acetate, but other halides produce a quenching effect similar to chloride and cannot be selectively measured by this method.¹²

Colorimetry uses the change in a color of a substance that can be observed with instrumentation or the naked eye to allow for detection of a substance. One of the mostly widely used colorimetric indicators is pH strips, which possess several halochromic compounds that change color in different pH regions to provide a method to accurately determine pH without the use of instrumentation. In the presence of specific molecules, the electrons, particularly those involved in π bonds, of the colorimetric indicator will change state, which causes a characteristic color change that can be used to show the presence of the specific molecule. Indicators also exist for many cations because of the color changes associated with partially filled d-orbitals in coordination complexes or through chelation type effects with other colored compounds.^{13, 14} Such an indicator does not exist for anions, which prevents the easy determination of the presence of aqueous anions.

In recent years, there have been many colorimetric anion indicators developed that elicit a color change through covalent or non-covalent interactions, such as hydrogen bonding and electrostatic attraction. Sulfide ions can be detected by a reversible pyrylium-thiopyrylium transformation that results in a distinct color change (Figure 2); however, as with fluorescent indicators, these covalent binding interactions rely upon the specific chemical properties of each anion and are not easily adaptable to other systems.¹⁵ There are several colorimetric anion indicators that interact through hydrogen bonding and electrostatic forces instead of covalent bonding; many of these indicators use protonated nitrogen groups such as guanidinium, pyrrole and amide residues for interaction with the targeted anion.^{16, 17} Unfortunately, most currently known colorimetric anion indicators become ineffective in more competitive, non-organic, solvents like DMSO and water where the solvent is able to compete as a hydrogen bond donor or acceptor.¹⁸ Thus, the discovery of a colorimetric indicator that is both selective to one or more specific anions and effective for analysis under aqueous conditions remains a significant focus of this group's work. Though a few examples exist, many more molecules must be synthesized and screened before a motif suitable for a comprehensive and systematic structure-activity study can be refined; therefore, the focus of this work is elaborating methods by which the parallel synthesis of large numbers of colorimetric molecular indicators can be accomplished.

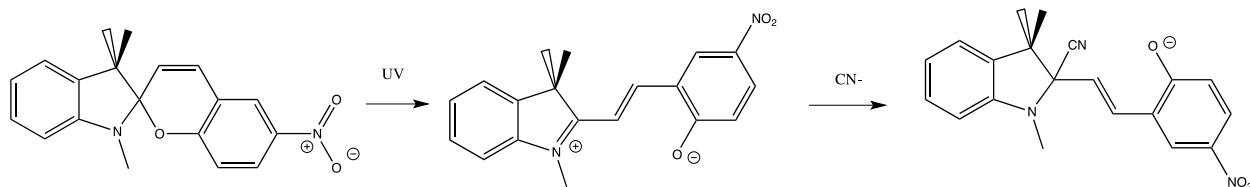


Figure 1. A coumarin-spiropyran conjugate created by Shiraishi *et al.* capable of detecting aqueous cyanide by a selective fluorescence enhancement with UV irradiation.⁹

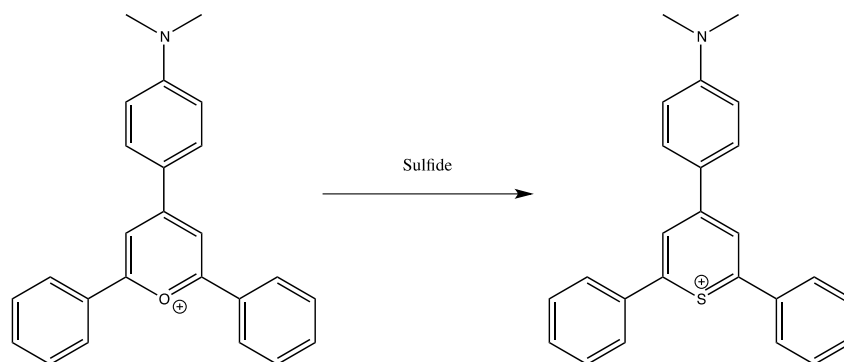


Figure 2. An aqueous anion indicator created by Jimenez *et al.* that shows a reversible color change through a pyrylium-thiopyrylium transformation with the addition of sulfide.

1.2 Review of the importance of azo dyes in colorimetry

Commercially available dyes were previously surveyed for potential colorimetric anion indicator properties by researchers in the Harris-Paul group, and through this investigation, the azo dye Sudan Black B (**1**), was discovered to show a pH-independent 30 nm shift in λ_{max} in the presence of bromide anion in DMSO solution (Figure 3).¹⁹ Aromatic azo dyes are named for the azo functional group (a nitrogen-nitrogen double bond, -N=N-), and they are strongly absorbing in the visible region of the electromagnetic spectrum due to their extended conjugation, making them extremely useful as textile stains and as colorimetric indicators.²⁰ Methyl orange (Figure 4) is a commercially available mono-azo dye, which is widely used as a colorimetric pH indicator owing to a color change between red and orange that occurs upon exposure to solutions of pH 3-4. Unfortunately, further studies of Sudan Black B and the mechanism of its color change have been complicated by several factors. As a lipid stain, Sudan Black B possesses very low water solubility, bringing into question its efficacy in the analysis of aqueous solutions. Furthermore, the commercially available dye preparations are not analytically pure, and it is not yet known how potential contaminants or side products may interfere with or participate in these

observed colorimetric effects. To date, efforts in the group to separate and identify the components of Sudan Black B have not been successful. Lastly, the availability of pure, single-molecule dyes is essential to further investigation of this selective color change; however, the synthesis of Sudan Black B analogues was not a viable option due to the synthetic challenges inherent in this molecule's bis-azo structure (containing two azo linkages) as well as the lack of experimental detail present in patent literature.

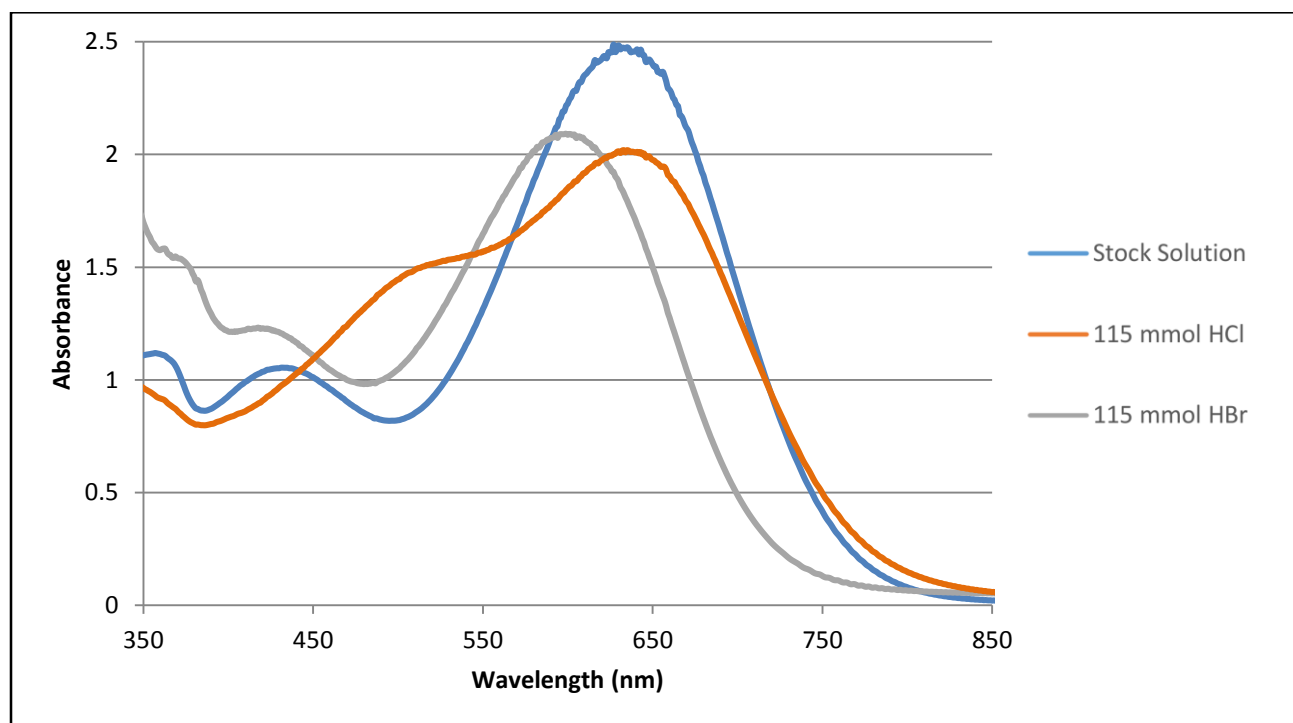
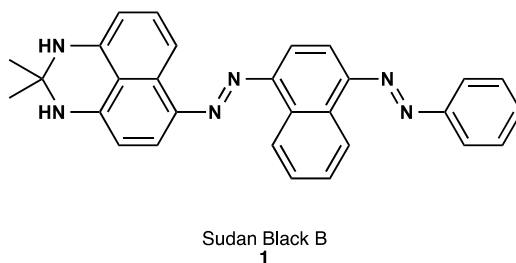


Figure 3. Sudan Black B, a bis-azo dye previously shown to change in the presence of bromide. UV-vis spectrum of Sudan Black B exposed to different concentrations of HBr and HCl (Reprinted from “The First Effective Method of Analysis for the Development of Ionochromic Azo Dyes for Aqueous Halide Detection” Honors Thesis by Heather Robison)¹⁹

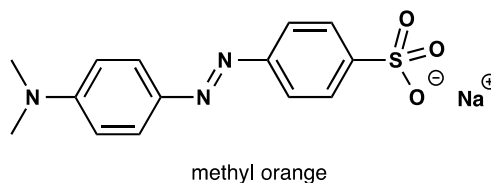


Figure 4. Methyl orange: a commonly used representative of the azo-dye class of molecules

Since the mechanism of the interaction of Sudan Black B with bromide is not known, a simplified dye structure was sought to probe the effect of different functional motifs on its colorimetric effect. Computational modeling performed by the Hadad Research Group at The Ohio State University, revealed both a significant negative partial charge and a significant LUMO on the central naphthalene ring of Sudan Black B.²¹ From these data, a simplified mono-azo structural motif was proposed by the Harris-Paul research lab (Figure 5) and has since served as the basis for their studies. The generalized structure was based around a single azo bond to simplify the structure of Sudan Black B; this bond was used to separate an electron-deficient aryl ring from another electron-rich aryl ring to imitate the charge density present in Sudan Black B. Though alternative methods of azo dye synthesis are possible, the most commonly used approach uses diazotization of an arylamine followed by azo coupling via electrophilic aromatic substitution (EAS) with an electron rich aromatic ring. Diazonium salts of aromatic amines are generated with sodium nitrite from a primary amine on an electron-deficient aromatic ring (Scheme 1). For the purposes of synthesizing Sudan Black B analogs, a diazonium cation is generated on an aniline ring substituted with an electron-withdrawing group (EWG), which will be referred to as the A-ring in this study and another aromatic ring structure substituted with an electron-donating group (EDG), which will be referred to as the B-ring in this study. Even using a single B-ring, there were many potential indicators that could be synthesized from the

selection of A-rings (Figure 5). To quickly create all of the candidate compounds, the OSU students enrolled in the Research Experiences to Enhance Learning (REEL) laboratory were asked to aid in their synthesis.

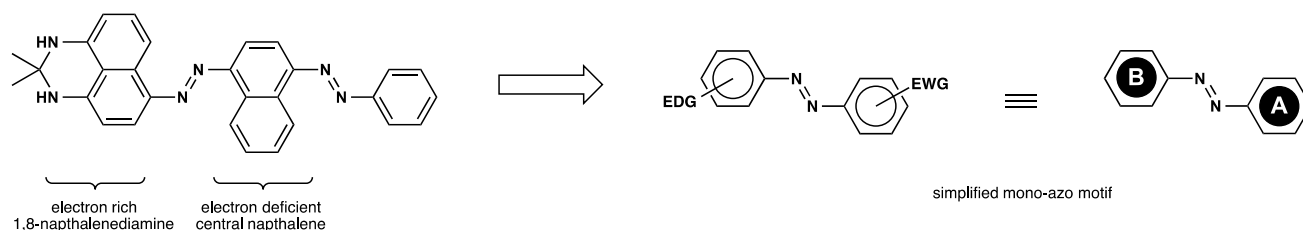


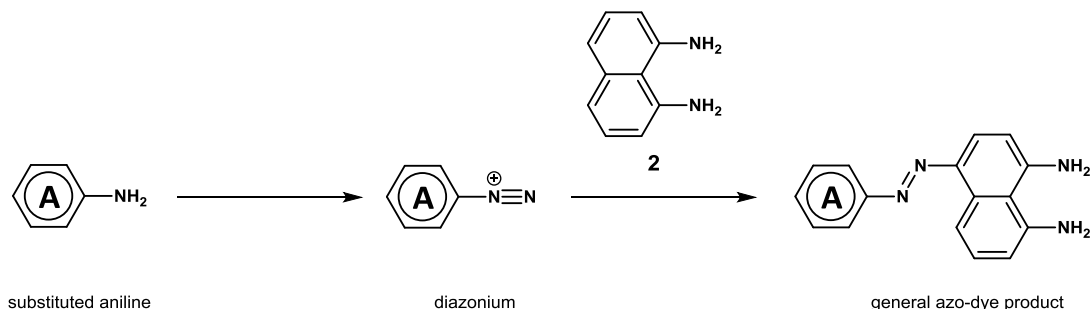
Figure 5. From Sudan Black B to the simplified mono-azo dye motif

1.3 The REEL program and identification of a potential indicator*

The Research Experience to Enhance Learning (REEL) program began as an initiative funded by the National Science Foundation to introduce undergraduate chemistry students to authentic research in the classroom. **The Search for a Selective Colorimetric Anion Indicator** project was conducted over five semesters of Organic Chemistry Laboratory II (Chem 255/2550) prior to the efforts outlined in this thesis. Participants in this research have worked to synthesize azo dyes using the process outlined in Scheme 1, where one of a selection of substituted anilines (A-ring component, Figure 6) was treated with sodium nitrite under acidic conditions, and the resulting aryldiazonium was coupled with 1,8-naphthalenediamine (ND, **2**) to yield a library of mono-azo dyes (Figure 7). These dyes were analyzed by UV-vis spectroscopy in the presence of chloride or bromide using a modified procedure based on that of the original dye survey protocol to determine if any of the compounds exhibited a colorimetric effect.¹⁹ The findings from these experiments were

* Worked conducted by students in the AU10, WI11, SU11, AU11, and WI12 sections of Chem 255/2550 at The Ohio State University under the supervision of Dr. Noel M. Paul and Dr. Chris S. Callam. Results are unpublished.

instrumental in shaping the course of this project; first, many of the synthesized products, like Sudan Black B, were not water soluble, which is not ideal for an indicator that would be predominantly used in aqueous solution. Second, a majority of the product mixtures could neither be separated to yield a single compound nor could they be completely identified. Despite these limitations, several groups of Chem 255 students in Winter 2011 found that the products from the attempted synthesis of the compound 3-ABA-ND (**3**, Figure 7) elicited a 30-nm blue shift in the presence of bromide and not chloride, similar to that of Sudan Black B. Unfortunately, the synthesis of 3-ABA-ND did not result in a pure product, so these positive results were again obtained from a mixture, leaving the identity of the molecule responsible for this effect unconfirmed. Inspired by the work of the REEL students, researchers in the Harris-Paul group devoted significant efforts to purify 3-ABA-ND free from crude contaminants. Ultimately, these efforts resulted in a molecule with the exact mass of 307.12 m/z [M+H] (consistent with that of 3-ABA-ND), which exhibited no colorimetric effects in the presence of bromide.¹⁹ Thus, new methods are needed to synthesize azo dyes in higher purity, and alternative strategies are needed to screen potential candidates for colorimetric effects more efficiently.



Scheme 1. General coupling strategy utilized by researchers participating in the REEL project.

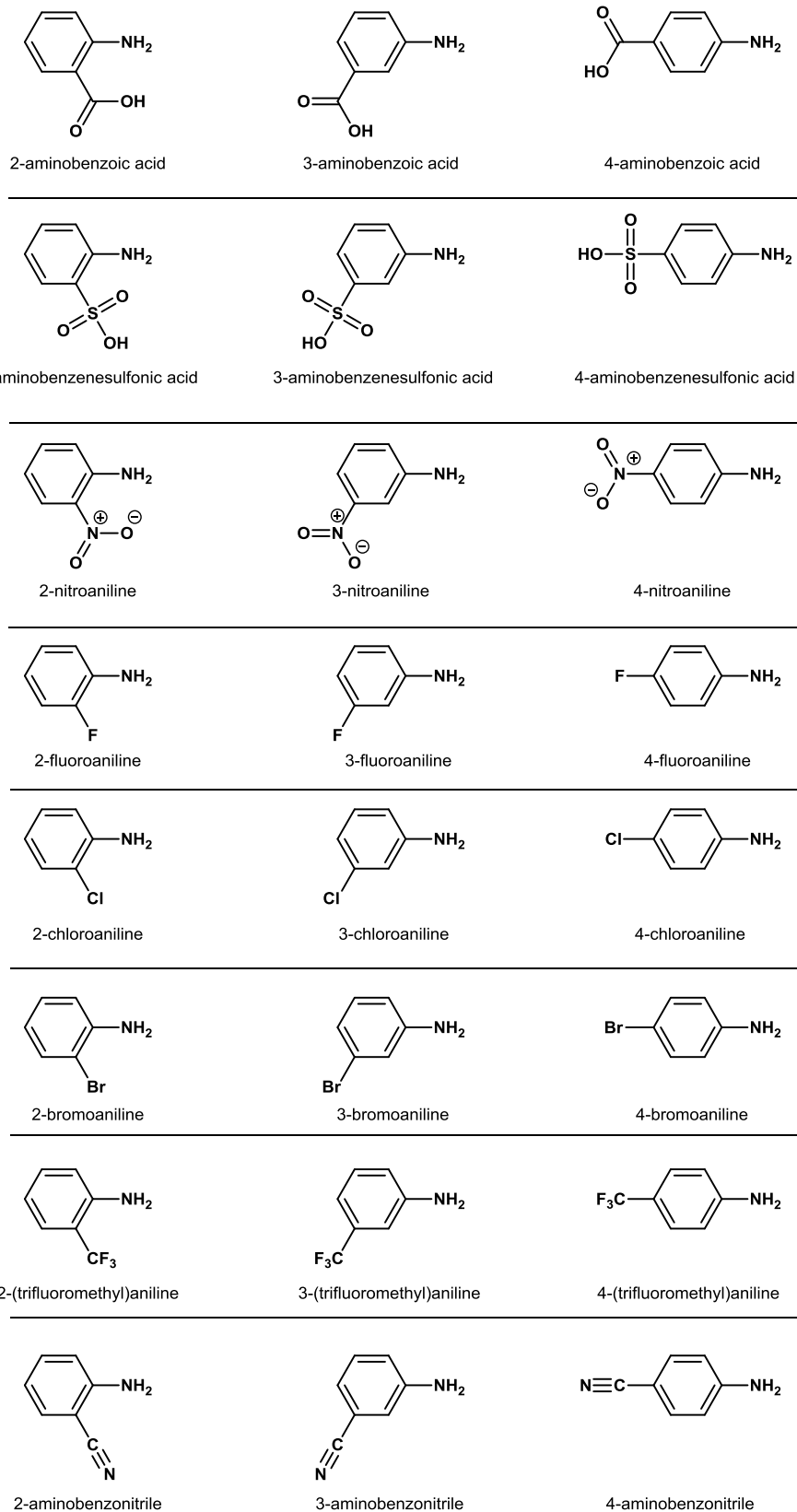
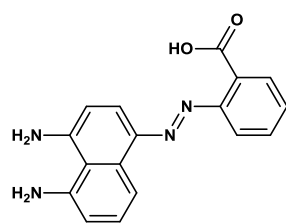
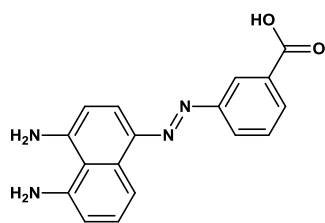


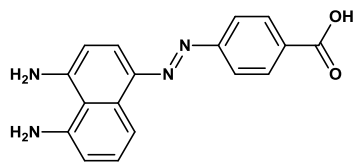
Figure 6. Choice of “A-rings” available to the students of the REEL project.



2-ABA-ND

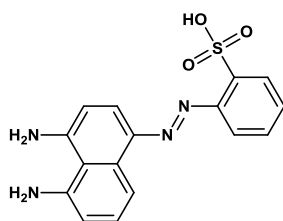


3-ABA-ND

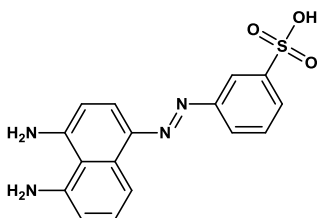


4-ABA-ND

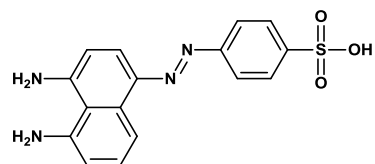
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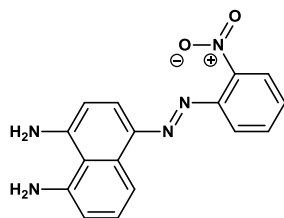
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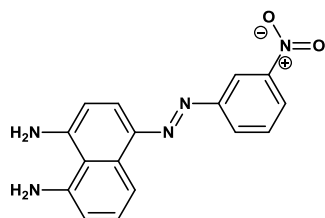
3-ABSA-ND



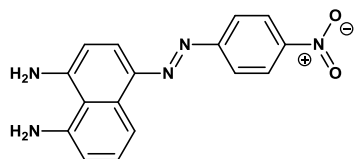
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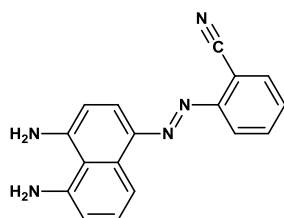
2-NA-ND



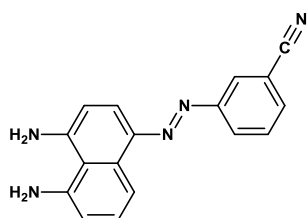
3-NA-ND



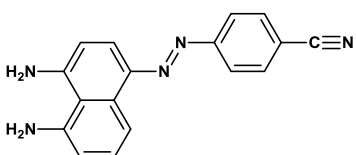
4-NA-ND



2-ABN-ND



3-ABN-ND



4-ABN-ND

Figure 7. Examples of azo dyes targets sought by the students of the REEL project.

1.4 Solid-phase synthesis and diazotization

A solid-phase synthesis was proposed as a solution to overcome the constant problems of contamination and low purity in azo dye synthesis, at the same time providing a new strategy with which to conduct colorimetric assays. However, diazotization and electrophilic aromatic azo coupling has not been documented on a solid-phase. Though historically used for the synthesis of oligopeptides, solid-phase synthesis methods have been used to create a variety of diverse targets using a range of reaction conditions.²² In solid-phase organic synthesis (SPOS), molecules are immobilized on a macroscopic solid support through strategic functional groups and then treated with reagents in solution allowing traditional organic transformation to take place on the surface of the support. Once the reaction is complete, the excess reagents, side-products and contaminants can be washed away, leaving the pure product covalently bound to the solid. In general, at the end of the total synthesis, the compound is cleaved from the solid and recovered using solution-phase techniques.^{23, 24}

Both the solid-phase itself and method of linking the relevant functional groups to the support are important steps in ensuring the quality of the synthesis. Most solid-phase syntheses take place on polymer resin beads that have been functionalized for synthesis. The solid-phase determines how the immobilized functional groups are arranged as a function of solvent; when a solid is not well swollen in solvents the accessibility of the functional groups decreases along with reaction rates.^{25, 26} In general, most solid-phase resins are made of unsaturated poly-aromatic polymers such as polystyrene cross-linked with another compound to increase its swelling capacity, and this resin type swells best in polar, aprotic solvents like NMP, DMF and THF.²⁷ One of the most widely used resins is

Merrifield resin (**4**), composed of divinylbenzene cross-linked polystyrene functionalized with benzylic α -chloro groups (Figure 8).

Linker strategy is the second key factor in a successful solid-phase synthesis. The linker strategy can affect many different aspects of the synthesis including the conditions to which the support is resilient, the method of cleavage and the structure of the final product. There are two main categories of linker strategies that are used in SPOS, integral and non-integral linkers. Integral linkers (exemplified by the Merrifield resin) are defined as the functionalized portion of the resin being part of the solid-support structure. Non-integral linkers generally have some sort of spacer molecules between the functional groups and the solid-support, allowing for better control over reaction speed and loading.²² Spacer molecules can be added between the solid-support and the chemically active groups to allow for better solvation of the functional groups and better solvent compatibility.

Linker strategies also dictate the methods of cleavage available. Traditionally, polypeptides were cleaved using HF due to its compatibility with common protecting group strategies; however, the risks associated with HF and the limited synthesis available with only one cleavage protocol led to the development of many other strategies, including linkers that are cleaved by base, strong electrophiles or nucleophiles, and light.²² One of the most notable linker strategies is the Wang linker, which is a *p*-alkoxybenzyl alcohol linker, which can be cleaved under less hazardous conditions of concentrated TFA due to the stability of the resin bound linker product. The Wang linker is generally attached to a peptide or molecule of interest through an ester linkage, which after cleavage results in a carboxylic acid group on the product. By using SPOS, previously inaccessible pure azo dye products can now be created through a relatively simple synthesis with minimal

purification, allowing for the easy creation and identification of potential aqueous colorimetric anion indicators.

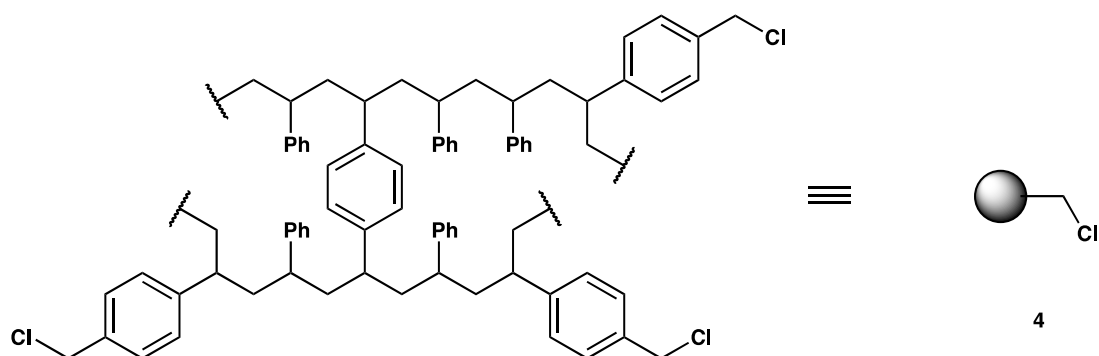


Figure 8. Chemical structure of Merrifield resin

Chapter 2: Methodology

2.1 Development of linker strategies

While there are many resins commercially available with varying affinities towards specific solvents and increased loading capacity (the quantity of a functional group present on a solid-phase per unit mass or area), Merrifield resin was chosen for its versatility, though it has not previously been used for azo coupling chemistry. Azo coupling reactions are commonly conducted as a one-pot synthesis with the generation of the diazonium ion in acidic medium followed directly by the addition of an electron-rich aromatic nucleophile; thus an appropriate linker had to be chosen in order to be resilient to those reaction conditions. Since the majority of commercial solid supports are cleavable under acidic conditions, a protocol for attaching a B-ring needed to be developed. While linker strategies that are acid-stable and cleaved under other conditions (base, UV radiation, etc.) exist, they are cost prohibitive and frequently require additional complications during

syntheses. Therefore, a cleavable-linker was not pursued and products were analyzed while immobilized on a solid support.

Although linking either the electrophile (A-ring) or the nucleophile (B-ring) of the azo coupling to the solid-support was possible, a more versatile solid support was created by functionalizing the resin with the nucleophilic azo coupling partner (B-ring). Since aromatic amines are being used as a nucleophile in this reaction, the aromatic rings are not the only nucleophilic portions of the molecule available for coupling. An unprotected (primary or secondary) amines can react with a diazonium ion to form a diazoamino compound, or triazene (**5**, Figure 9)²⁸. Since this is an undesirable side product, attachment of the B-ring to the solid support acts as a protecting group and prevents side reactions.

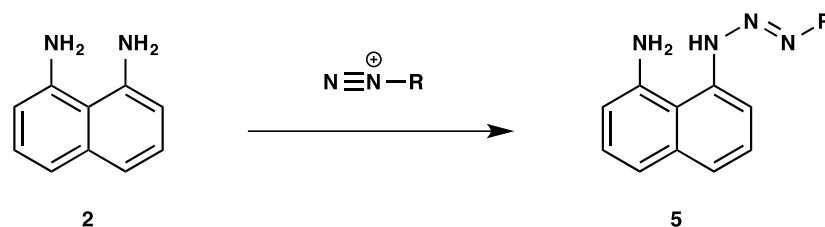
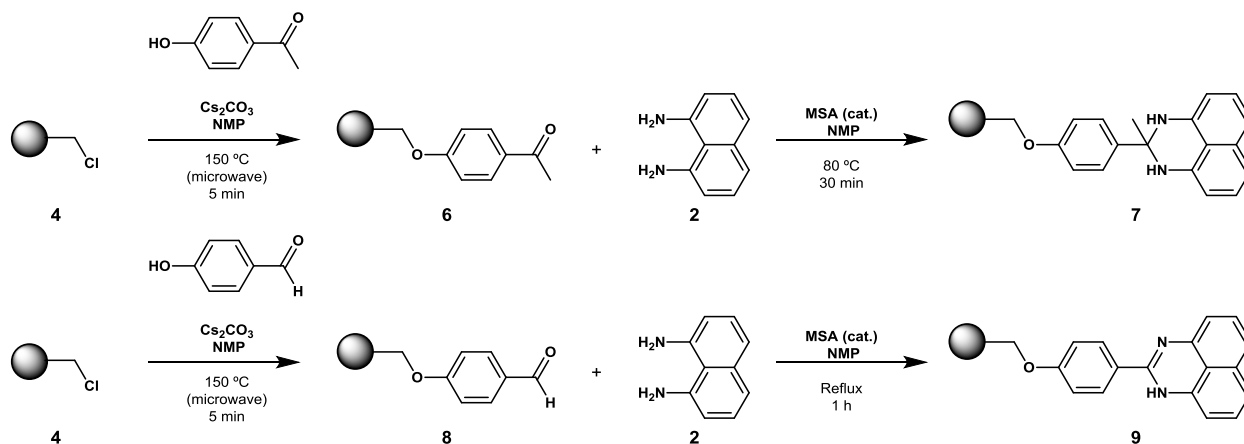


Figure 9. Diazoamino group formation as a result of an azo coupling side reaction between naphthalene-1,8-diamine and a diazonium salt.

Three potential protocols were developed for attachment of the nucleophile to the solid-phase. The first two protocols involved connecting **2** to **4** via an aminal (**7**) or perimidine (**9**) linkage formed through a condensation with an acetophenone or benzaldehyde, respectively (Scheme 2). These phenolic hydroxyl groups were attached to **4** using a microwave- assisted $\text{S}_{\text{N}}2$ coupling in NMP due to its high dielectric constant, loss tangent (which describes how well a material dissipates electromagnetic energy into heat) and resin swelling to yield **6** and **8**. Cesium carbonate was used as a base in these reactions due to its high solubility in NMP compared to other salts. Concurrent to this work, Albanese

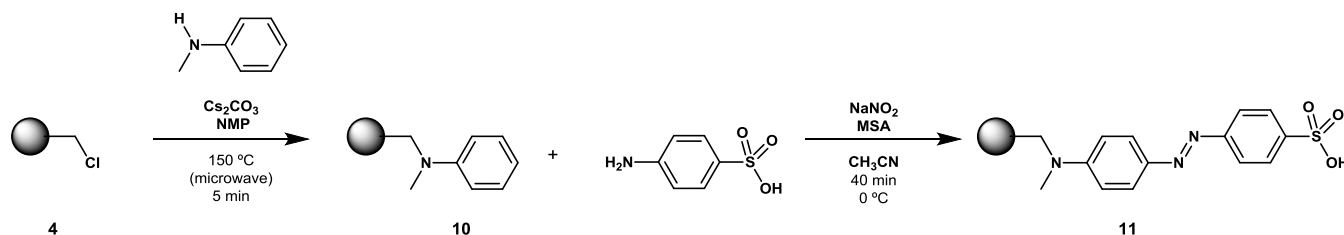
(2012), another researcher in the Harris-Paul group demonstrated that azo dyes based on the perimidine nucleophile do not elicit an anion-selective color change, and cannot be used to create bis-azo dyes, thus this linker strategy was abandoned.²⁹ The aминаl linker was subjected to strong acid conditions in high heat to verify its stability and no significant cleavage was observed. Despite these linkers having available secondary amines, the steric hindrance of the methyl and aryl groups was hypothesized to slow the formation of diazoamino side products. The last linker strategy was a direct attachment between the integral linker of the Merrifield resin and nucleophile through a microwave-assisted S_N2 coupling; only secondary amines could be coupled by this method to prevent formation of the diazoamino product.³⁰ This method was explored using *N*-methylaniline (NMA) as the nucleophile to afford a tertiary amine stable to diazotization conditions.



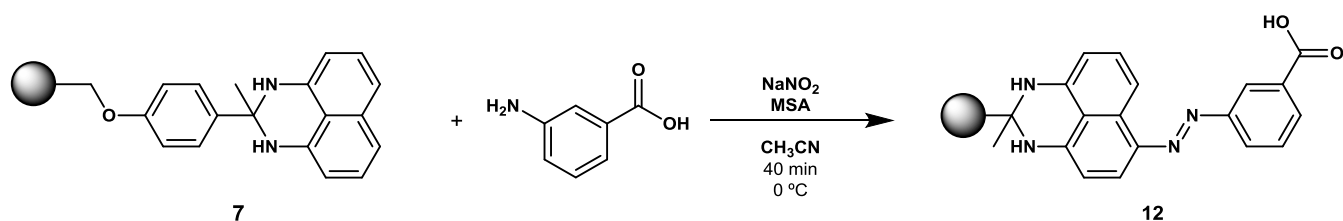
Scheme 2. Synthesis of aминаl (7) and perimidine (9) linkers on Merrifield resin (4) by a microwave-assisted S_N2 coupling and subsequent acid-catalyzed condensation.

2.2 Synthesis of Methyl orange and 3-ABA aminal on Merrifield support

Azo dyes were synthesized from the solid-supported aminal (**7**) and NMA resin (**9**) using 3-ABA and 4-aminobenzenesulfonic acid (4-ABSA) as the electrophiles, respectively (Scheme 3, Scheme 4). Under these conditions, acetonitrile was effective in swelling the resin and no additional swelling steps were necessary prior to the reaction. The reaction products could not be analyzed by NMR because they are bound to the insoluble support, so product formation was determined by changes in functional groups that were characterized by diffuse reflectance UV-vis-NIR spectroscopy. The azo dye formed by the reaction of NMA and 4-ABSA is the solid-supported equivalent of methyl orange, a colorimetric acid indicator (Scheme 4); this resin was exposed to acid and showed the same change in color as the commercially available indicator. Unfortunately, no colorimetric effect was observed when the 3 ABA aminal (**12**) was subjected to halide



Scheme 3. Synthesis of methyl orange on Merrifield resin (**11**).



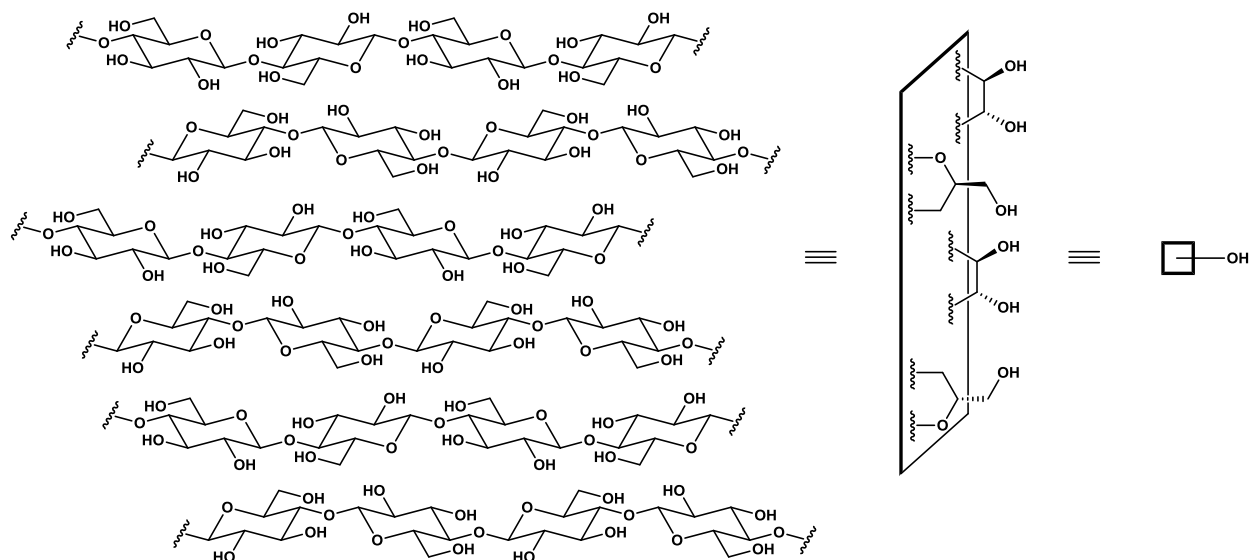
Scheme 4. Synthesis of 3-ABA aminated azo dye on aminated resin (12).

2.3 Functionalization of cellulose and facilitation of the Autumn 2013 REEL lab[†]

Having demonstrated a method for the synthesis of azo dyes on a solid support, as well as showing that solid-supported 3-ABA-ND (**3**) analogues, like **12**, do not possess a colorimetric shift in the presence of bromide, it again became prudent to synthesize and screen a large number of potential indicators to determine which functional motifs were worth exploring. To this end, the solid phase methodology was modified to facilitate the successful synthesis of a library of uncontaminated novel azo dyes by the students participating in the Autumn 2013 REEL lab; this endeavor resulted in new challenges to overcome. A new, less expensive solid phase needed to be developed because the Merrifield resin was cost prohibitive for the use by 100 undergraduate students in the REEL lab. Cellulose was chosen as the solid-support for several reasons (Figure 10). First, the paper was significantly cheaper than the Merrifield resin, even considering the cost of necessary reagents for functionalization. Second, the paper was resilient to most reaction conditions and retains most of its rigidity even after chemistry had been carried out on its surface. Lastly, using a planar instead of a spherical solid phase allowed for use of SPOT synthesis of an array of similar compounds for easy screening.³¹ SPOT synthesis was developed as a

[†] See Appendix 2 for list of all supervising staff, organizers and participants

method of synthesizing libraries of small proteins simultaneously for a fraction of the cost of traditional methods by spotting small portions of reagents on functionalized cellulose membranes. This synthesis method has also been adopted by organic chemists for the simultaneous synthesis of an array of similar molecules that can be easily screened for physical or chemical properties, such as colorimetric indication.²⁴

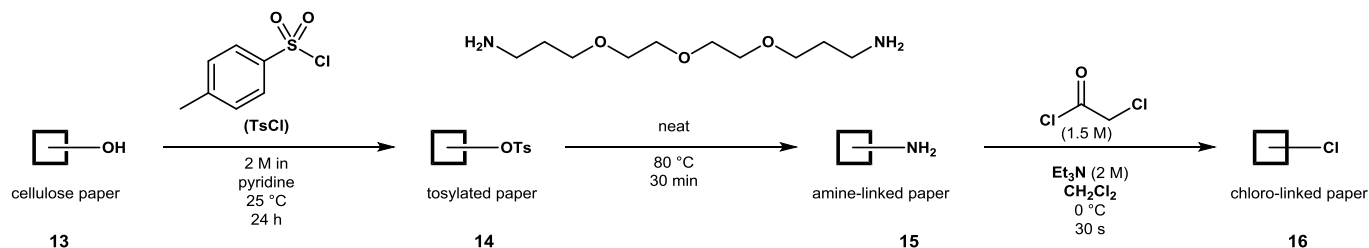


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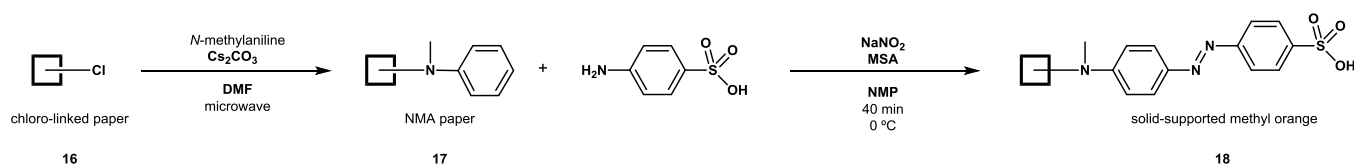
Figure 10. Molecular view and abbreviation of cellulose chromatography paper (13)

Just as the cellulose in SPOT synthesis has to be functionalized to accommodate polypeptide synthesis, it was necessary to develop a method of attaching the important functional groups to the solid phase to carry out the azo coupling. A procedure adapted from literature was used to functionalize cellulose chromatography paper with a diamine spacer unit (**15**), which was subsequently amidated with α -chloroacetyl chloride to provide a reactive terminal α -chloro functional group similar to that of the Merrifield resin (**16**) (Scheme 5).³² To demonstrate the ability of the functionalized cellulose membrane to be

used for synthesis of novel azo dyes, methyl orange was synthesized on the support and shown to demonstrate the same colorimetric shift as observed on the Merrifield resin and in solution (Scheme 6); however, there was not a large enough quantity of product formed for analysis by UV-Vis-NIR.



Scheme 5. α -Chloro functionalization of cellulose membrane with addition of diamine spacer unit.



Scheme 6. Synthesis of methyl orange using α -chloro functionalized cellulose.

Small arrays of solid-supported azo dyes were synthesized on a 5-cm² sheet of α -chloro functionalized cellulose paper (16) by the students in the undergraduate organic chemistry lab during the Autumn semester of 2013. These dyes were synthesized using a generalized diazotization procedure from several sets of A- (Figure 11) and B-rings (Figure 12) (Scheme 7). The students functionalized the support with assigned B-rings using the microwave-assisted coupling previously outlined in the synthesis of methyl orange (Scheme 6). The resulting paper was spotted with four different A-rings according to the diagram (Figure 13); these arrays were submerged in a solution of diazotizing agent to

create an array containing 4 unique azo dyes (Figure 14). Synthesized dyes were subjected to halide trials and analyzed using a UV-vis reflectance probe.

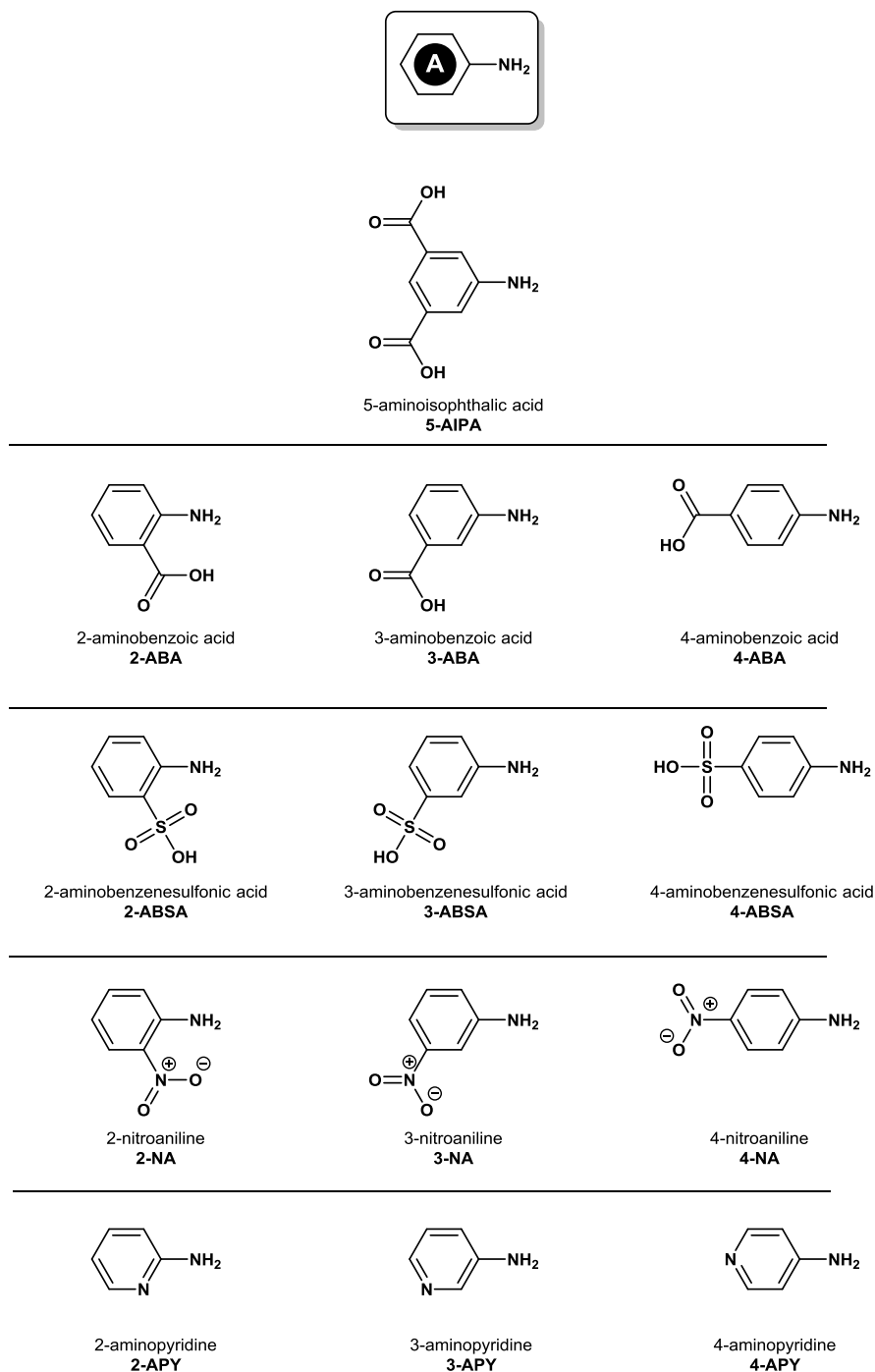


Figure 11. A-rings used in the Autumn 2013 REEL project

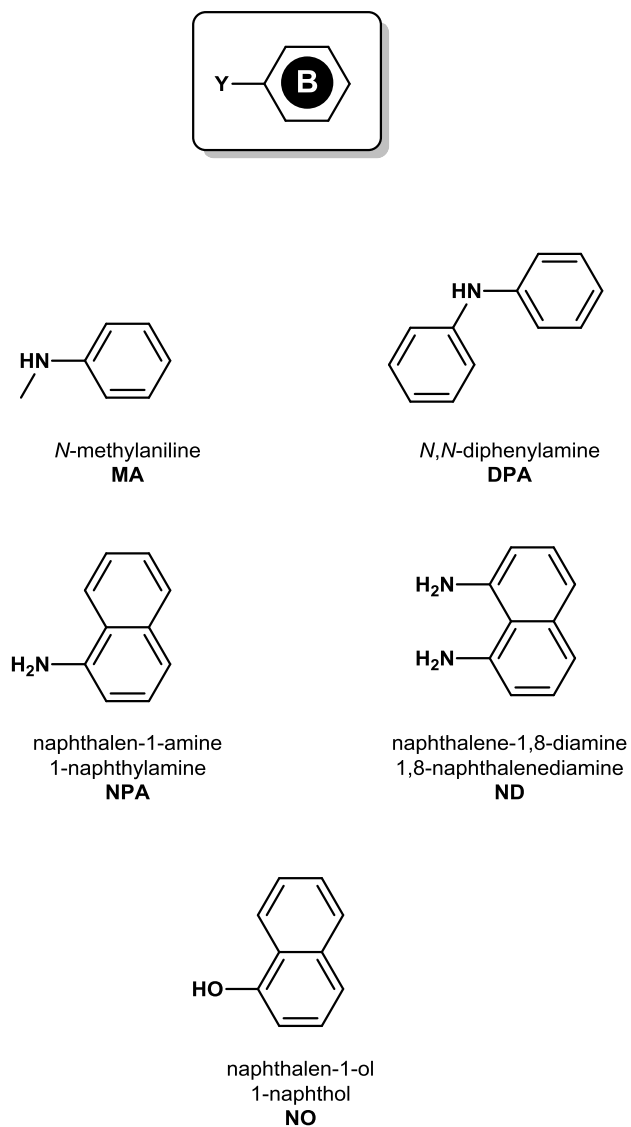
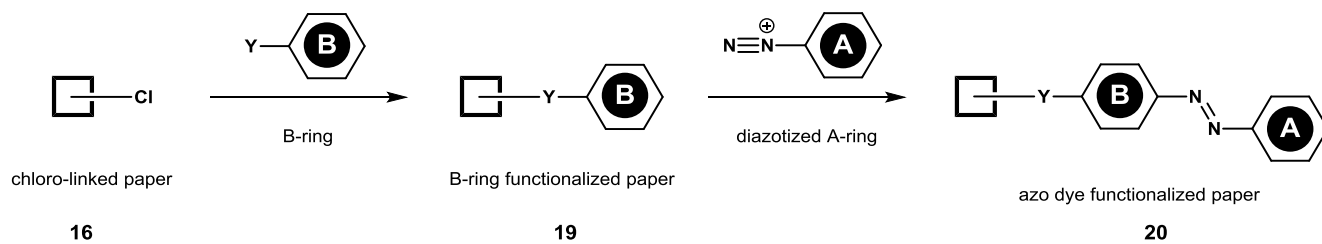


Figure 12. B-rings used by students in the Autumn 2013 REEL project



Scheme 7. Generalized diazotization procedure carried out on functionalized cellulose

test strip number:	1	2	3
blank (B-ring only)			
3-ABA	●	●	●
A-ring #2	●	●	●
A-ring #3	●	●	●
A-ring #4	●	●	●

Figure 13. Spotting diagram provided to students for the Autumn 2013 REEL project to create solid-supported azo dye arrays.

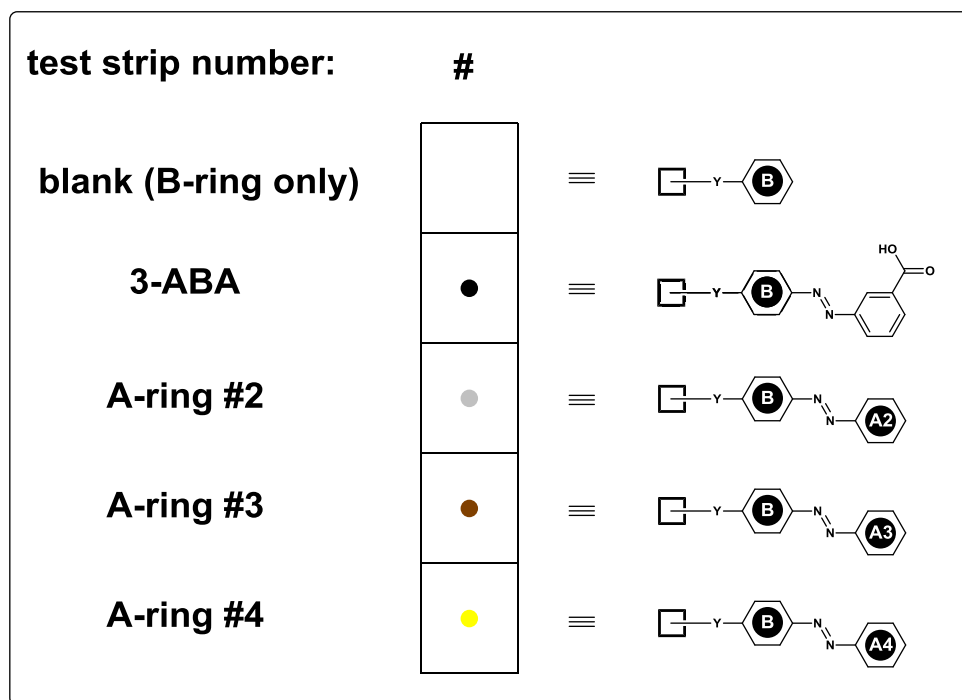
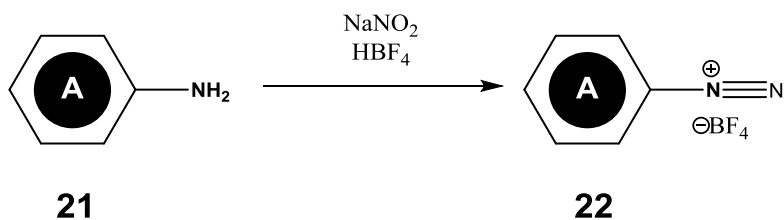


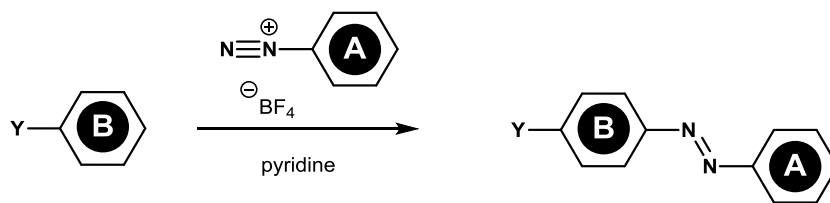
Figure 14. Molecular view of completed azo-dye array.

2.4 Refinement of diazotization protocol

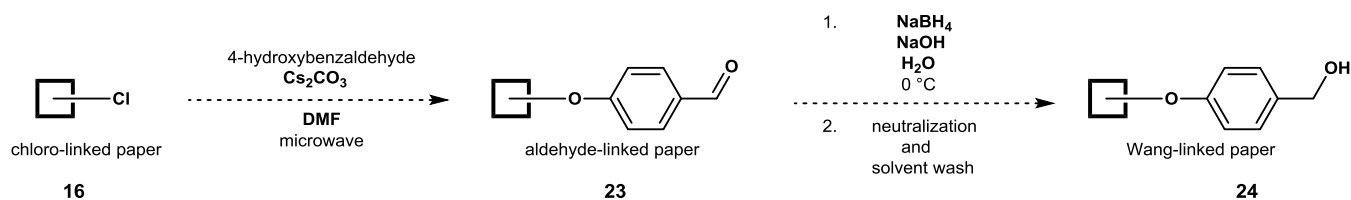
A new method was developed using a diazonium tetrafluoroborate salt synthesized from an A-ring, sodium nitrite, and tetrafluoroboric acid, which was isolated as a surprisingly stable diazonium tetrafluoroborate salt (Scheme 8). The salt was combined with the B-ring with pyridine to yield pure azo dyes after acid-base extraction (Scheme 9)²⁸. This new methodology had potential to improve results for two reasons: first it eliminates potential side reactions occurring because of excess sodium nitrite, and second, many of the nucleophilic compounds utilized in the coupling are basic causing them to protonate under previous reaction conditions. Additionally, it allows for the implementation of the acid-labile Wang-linker for the solid-phase synthesis of cleavable azo dyes (Scheme 10). This new methodology was used to synthesize novel azo dyes in solution (**27**) and on a cleavable support (Scheme 11).



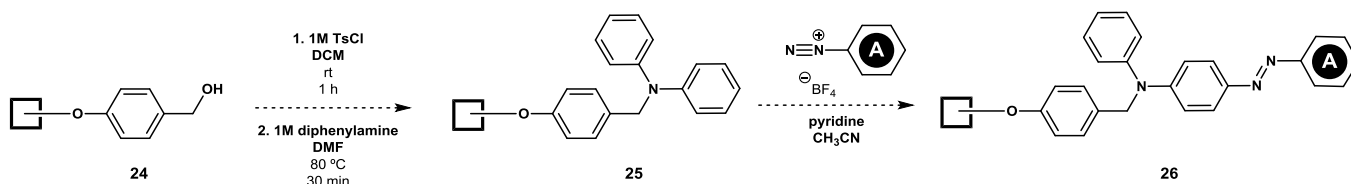
Scheme 8. Diazonium tetrafluoroborate salt synthesis from A-rings



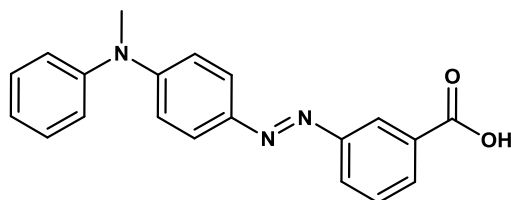
Scheme 9. Generalized azo coupling with B-ring via diazonium tetrafluoroborate salt, catalyzed by pyridine.



Scheme 10. Synthesis of Wang linker from 16 by microwave-assisted $\text{S}_{\text{N}}2$ coupling of 4-hydroxybenzaldehyde similar to Scheme 6 and subsequent reduction to 24.



Scheme 11. Synthesis of a Wang-linked azo dye utilizing the generalized diazonium tetrafluoroborate salt azo coupling procedure.



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2.5 Diffuse reflectance UV-vis-NIR spectroscopy

Diffuse reflectance UV-vis-NIR spectroscopy is a powerful tool for the analysis of insoluble materials that are poorly suited to other methods of analysis. This instrument scans a 2200 nm region, allowing for the simultaneous determination of functional groups present and the measurement of colorimetric changes on an insoluble material.³³ Infrared spectroscopy (IR) extends from the red edge of the visible spectrum (~ 700 nm) all the way up to 1 mm and includes three regions, near- (NIR), mid- (MIR) and far- (FIR) infrared. The energies in the infrared spectrum correspond to the vibrational energies of covalent bonds, which allow these spectroscopic regions to be useful in determining the presence of specific functional groups in a molecule. The NIR region encompasses between 800 nm and

2500 nm and corresponds to combination vibrational frequencies as well as the third and fourth overtones of the fundamental IR absorptions found in the MIR regions. These frequencies result in complex spectra that can often be difficult to analyze. While NIR does have its disadvantages, it is more useful for analysis of solid phases than MIR because it is able to penetrate more deeply into a solid and provide higher quality results.³⁴

A Cary 5000 UV-Vis-NIR spectrophotometer equipped with an integrating sphere for diffuse reflectance measurements was utilized to analyze polystyrene resin- and cellulose-supported intermediates and products. Stretches of importance were assigned and used to suggest the identity of the compounds attached to the solid support. UV-Vis NIR by itself cannot be used to identify a compound, so NMR and HPLC-MS-TOF were utilized, where applicable, to better understand the structures of the compounds present.³⁵

Chapter 3: Results and discussion

3.1 Analysis of polystyrene resin-bound products

The polystyrene resin-bound methyl orange (**12**) was shown to have a colorimetric shift of λ_{max} from 600 to 650 nm similar to that of the commercially available acid indicator (Figure 15). A colorimetric shift is seen at 600 nm. Azo-bond stretch was observed as a shoulder at 1500 nm. Disappearance of a peak at 1100 nm with the addition of MSA was attributed to the protonated of the sulfonate residue. These results demonstrated that these types of colorimetric changes can occur with azo dyes on a solid support, just as they can when dissolved in solution. A similar experiment was conducted on a functionalized cellulose membrane prior to the 2013 Autumn REEL lab and a similar effect was visually observed; however, there was not enough product to test by these methods.

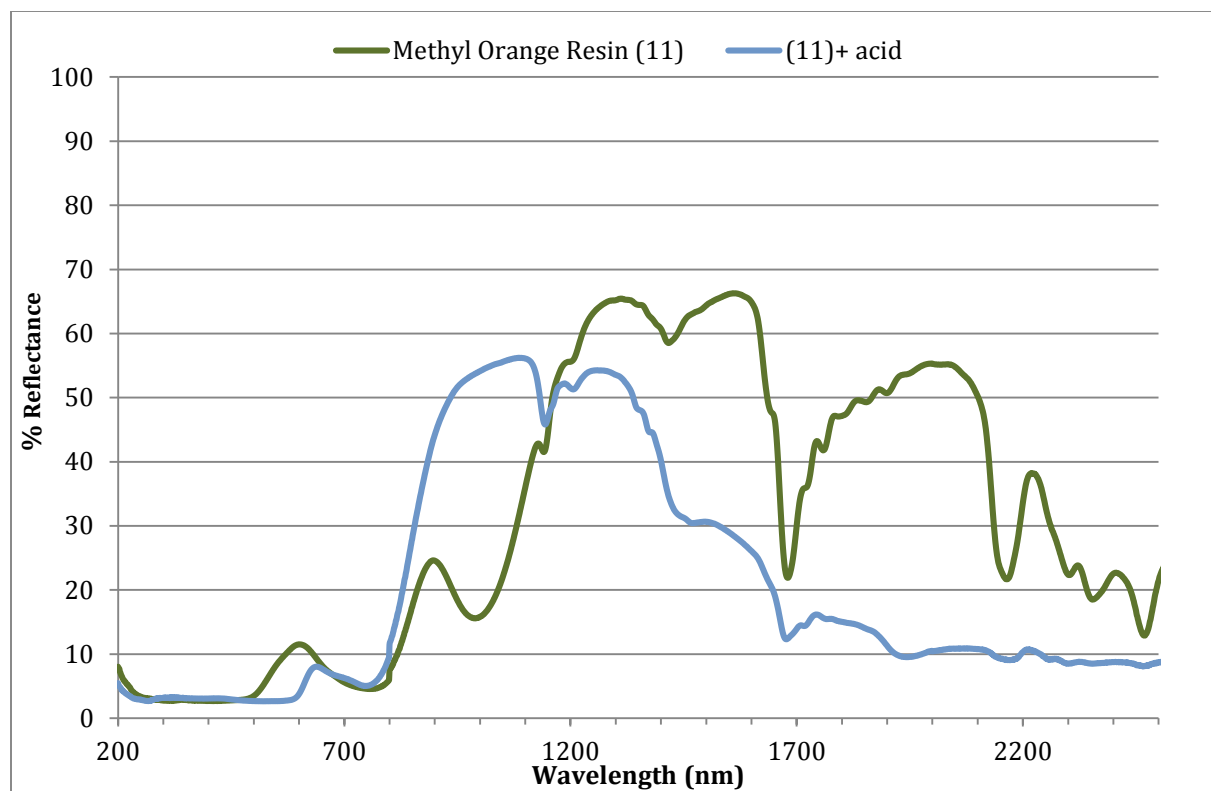


Figure 15. UV-vis-NIR of resin-supported methyl orange (12) before and after exposure to methanesulfonic acid (MSA).

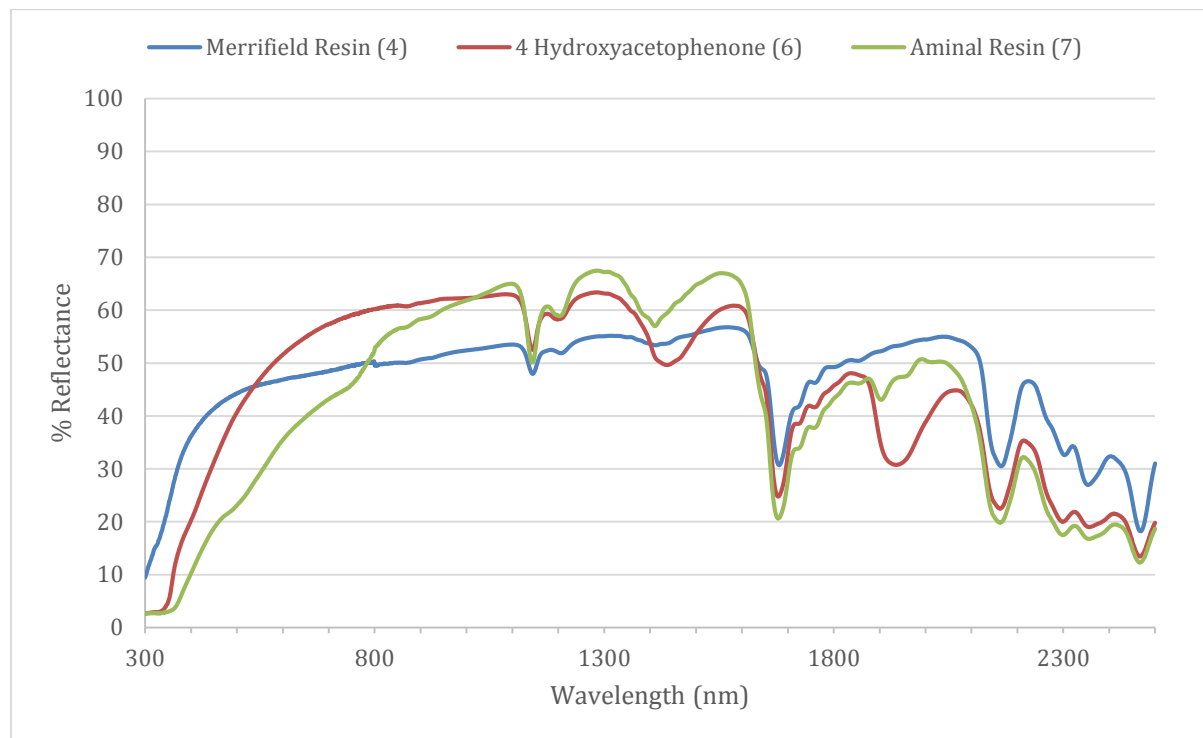
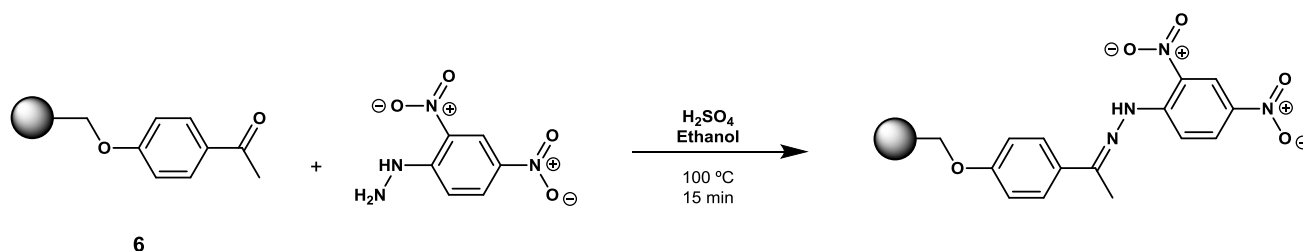


Figure 16. UV-vis-NIR of 7 and precursors, 6 and 4.

The aminor resin (**7**) was synthesized and analyzed by the reported technique (Figure 16). The peaks observed at 1850 and 2100 nm in **6** that are not present in **4** are characteristic of the second overtone of an aromatic carbonyl stretch, with peaks characteristic of the third overtone being observed at 1300 and 1600 nm.³⁶ These observations suggests the 4-hydroxyacetophenone coupling was successful. The exact IR stretches of an aminor residue are not well documented in the literature; therefore, it was difficult to confirm without solution-phase synthesis and characterization of the product that an aminor residue formed on the support. Furthermore, the amine stretches at 2150 nm, which should be visible are covered by stretches found in the stock resin. The formation of the aminor residue (**7**) is suggested by the disappearance of the stretch at 1910 nm compared to **6**. This attribute indicates the disappearances of the carbonyl residue, which would not be present in **7**. It is possible that the aminor condensation did not proceed completely and the nucleophile is attached to the resin by an imine bond instead, which could be the cause of the small stretch at 1900 nm. To support the formation of the aminor resin, **6** and **7** were analyzed with Brady's reagent to detect the presence of a ketone (Scheme 12); by this method, **6** was confirmed to contain a ketone residue, which was absent in **7**. Brady's reagent is not as reactive towards imines as it is towards ketones and aldehydes, so a false negative could have occurred if an imine was formed instead of an aminor. Formation of an imine could inhibit the synthesis of a pure azo dye product in the following steps due the unintentional synthesis of a diazoamine product or acid-mediated cleavage from the solid support; nevertheless, diazotization was attempted and the products were characterized by UV-vis-NIR.

Compound **12** was synthesized via diazotization of 3-aminobenzoic acid and coupling to **7** according to the noted procedure. There was an azo functionality identified in the NIR region, by the shoulder at 1565 nm in MSA (Figure 17). Resin product **12** was subjected to halide testing to determine the presence of any colorimetric effect in the presence of halides (Figure 18), unfortunately no effect was observed.

Although **12** demonstrated a lack of colorimetric effects in this experiment, sufficient evidence for the incorporation of azo dye structures onto the solid support to warrant the scale up of this study in a REEL experiment as part of the Autumn 2013 Chem 2550 laboratory. A new solid support was developed according to an adapted literature procedure and success was monitored using a combination of UV-vis-NIR spectroscopy and colorimetric indication.³²



Scheme 12 Brady's reagent was used to determine the presences of aldehyde and ketone residues on the solid support by development of a bright orange color.

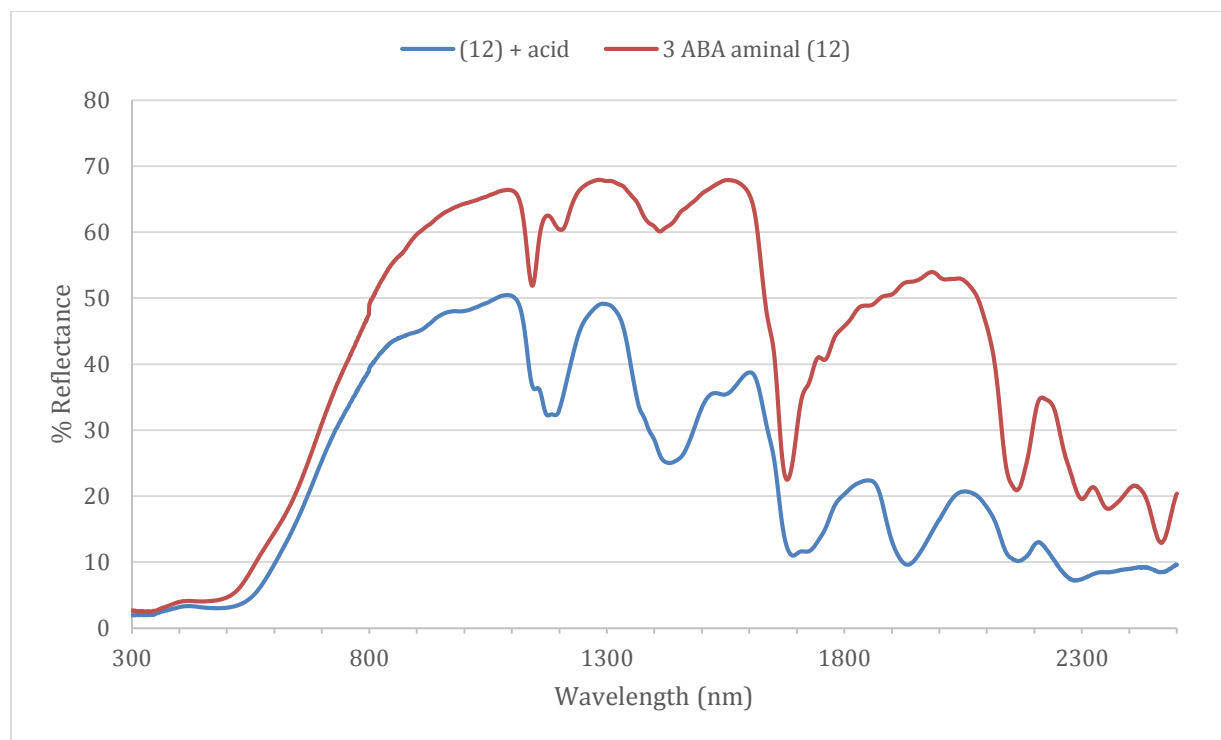


Figure 17. UV-vis-NIR of 3-ABA Aminoal Resin.

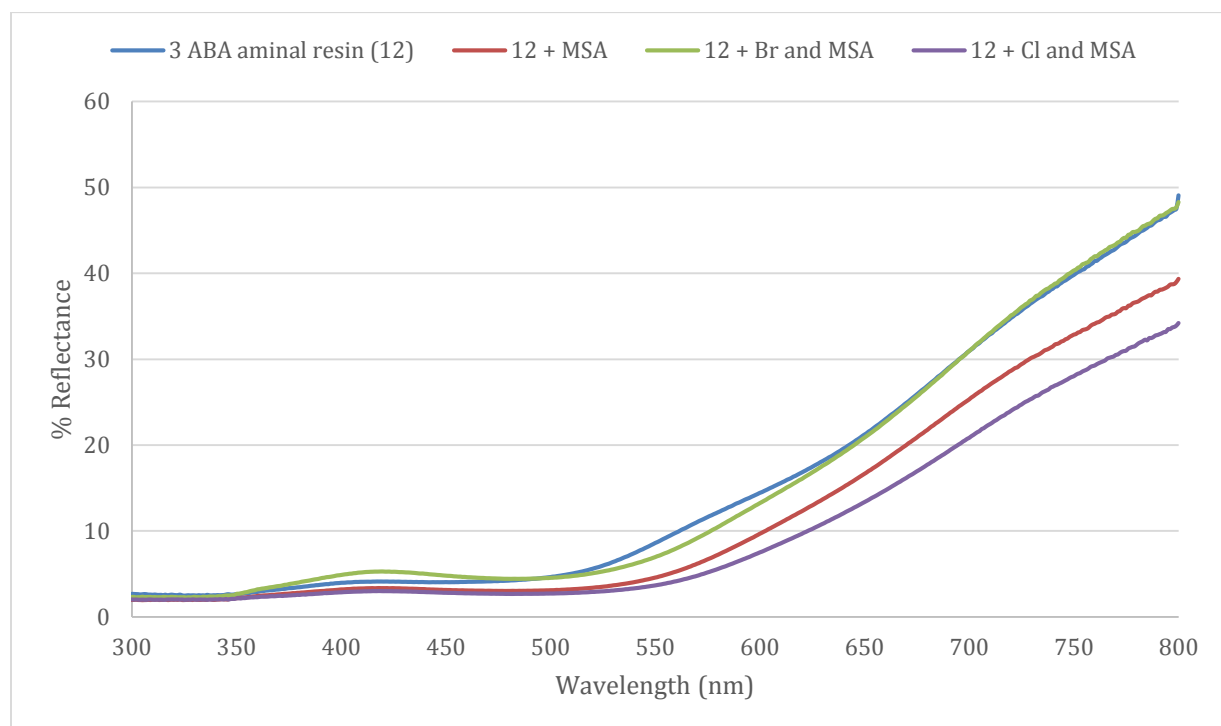


Figure 18. UV-vis spectroscopy halide trials on 3-ABA aminoal resin (12). There is no colorimetric shift present with the addition of acid, chloride or bromide.

3.2 Analysis of cellulose-bound products

Cellulose paper was activated for functionalization by a tosylation procedure (Scheme 5). While the progress of this reaction could not be directly monitored, the loading was quantified by the succeeding reaction and used to optimize the reaction time for the tosylation (Figure 19). Following the tosylation, an amine spacer molecule was inserted, though NIR spectra obtained for **15** did not conclusively show the presence of an amine. Ninhydrin test was used to quantify the loading of the amide linker using its extinction coefficient. The next step, the amide coupling utilizing α -chloroacetyl chloride, was confirmed by a negative ninhydrin test following the reaction. Additionally, the presence of the α -chloro group was confirmed by reaction with fluorescein producing a bright green-yellow fluorescence.²³

Sheets of α -chloro-functionalized cellulose chromatography paper (**16**) were given to the students of the Autumn 2013 Chem 2550 REEL lab, who attempted to functionalize the support for the synthesis of new azo dyes (Scheme 7). Few students observed an indicative color change when exposing their cellulose samples to the diazotization solution. These putative azo dye arrays were then subjected to halide trials and analyzed with the Ocean Optics USB4000 diode array equipped with a fiber optic diffuse reflectance probe. The full visible spectra were taken and analyzed for presence of a colorimetric shift; there was no clear evidence of colorimetric effects in any of the gathered data.

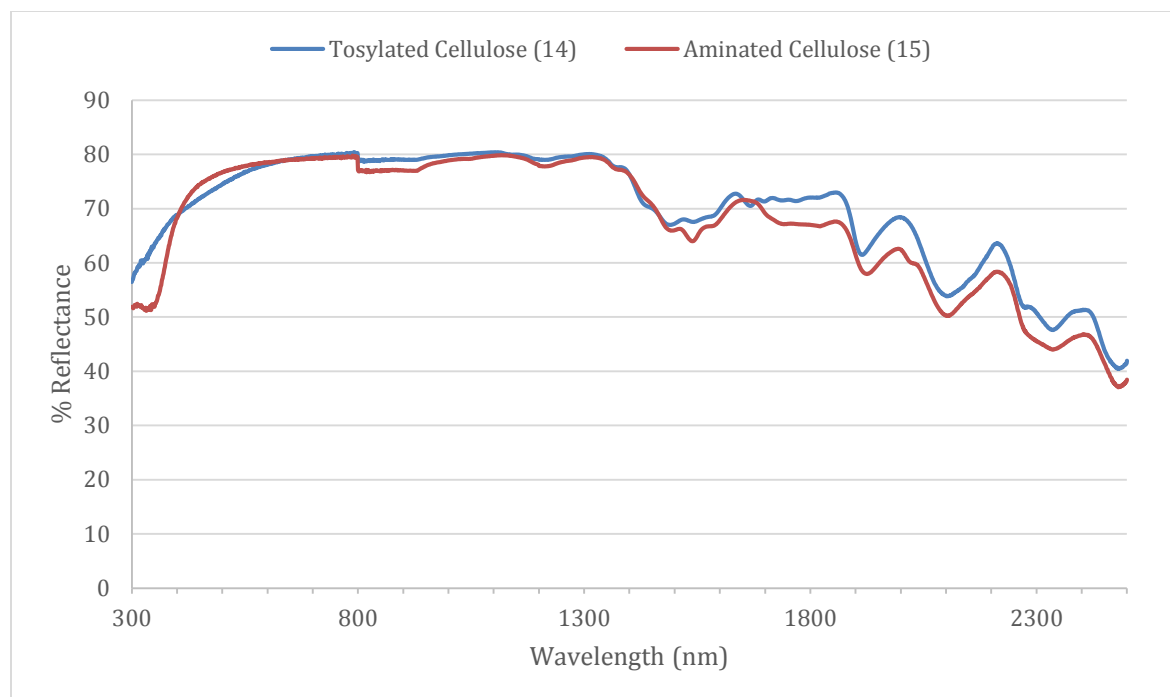


Figure 19. UV-Vis NIR of tosylation and amination of cellulose membrane.

To confirm the negative results of the Autumn 2013 REEL project experiments, the synthesis of an acid-cleavable diphenylamino functionalized cellulose was conducted (Scheme 10, Scheme 11). The intermediates of the Wang-linker cellulose functionalization were analyzed using UV-vis-NIR. The spectra of **(23)**, **(24)** and **(25)** were identical, which may suggest that the Wang-linker functionalization was unsuccessful (Figure 20). Synthesis of **(26)** was not attempted due to the absence of an adequate support. These data supported the idea that coupling of an aromatic amine to the cellulose support was not successful, and likely the reason for the lack of observed color during the REEL experiments as well.

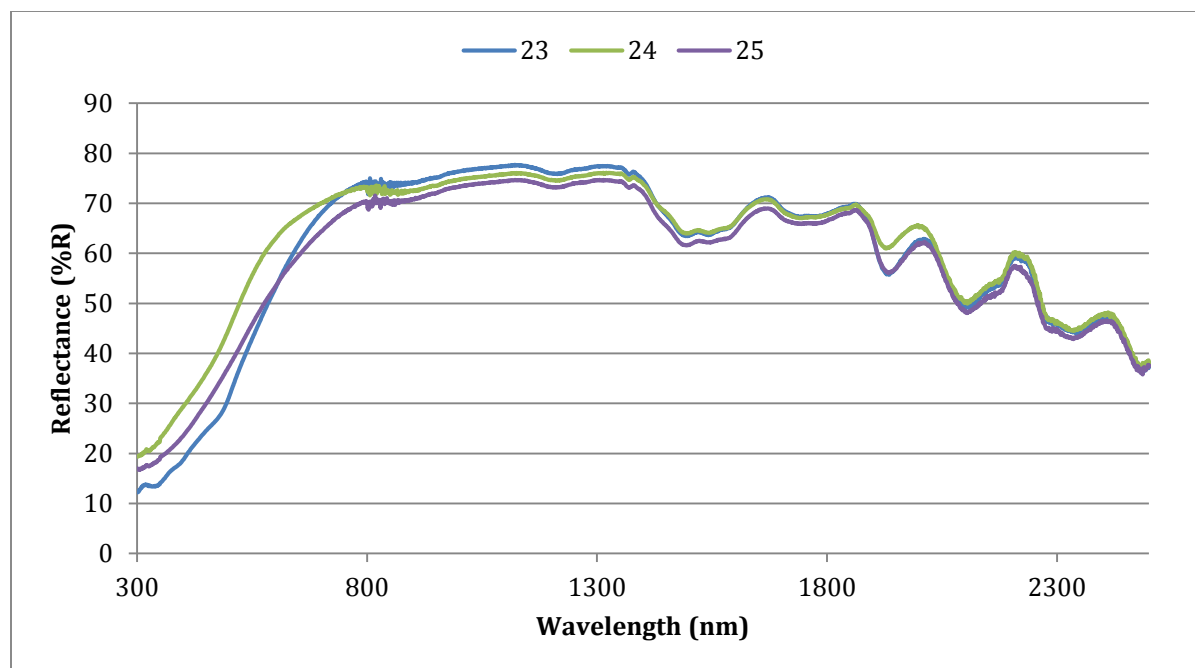


Figure 20. UV-Vis NIR of Wang-linker synthesis (Scheme 10). No change in spectra between steps indicates that no reaction has occurred.

Chapter 4: Conclusion

This project was unsuccessful in the design of a colorimetric indicator for aqueous anions; however, this researcher's efforts have clarified many of the problems that have previously plagued this synthesis. This researcher has developed a solid-support and a methodology with the capability of simultaneously synthesizing a large number of azo dye molecules, making it easier to probe which functional groups show a colorimetric effect and to quickly narrow down the possibilities. Additionally, if the azo dye structure is abandoned as a potential halide indicator, this methodology, with some modification, can be applied to the synthesis of many different types of molecules. Additionally, this researcher has demonstrated a methodology for azo coupling under basic conditions to provide ((*E*)-3-((4-(methyl(phenyl)amino)phenyl)diazenyl)benzoic acid, **27**), and this methodology can be applied to the synthesis of a wide variety of azo dyes while decreasing

the potential for troublesome contamination. Likewise, a method for azo coupling under basic conditions allows for investigation of the previously unusable acid-sensitive linker strategy.

Although synthetic issues thwarted the identification of new colorimetric anionic detectors during the Autumn 2013 REEL project, solid support-bound methods continue to hold promise as tools for the synthesis and study of novel azo-dye arrays. While there has been success, both documented in the literature and in work outlined in this thesis, with the microwave-assisted S_N2 coupling of an aryl nucleophile to a solid support, it is possible that the current procedure for this coupling is too harsh for use on the cellulose membrane or that it will continue to be unsuccessful for the nucleophiles chosen.³⁰ Success with this reaction has only been documented with monocyclic ring structures (*N*-methylaniline and 4-hydroxybenzaldehyde) and many of the chosen nucleophiles for the microarrays include more bulky and electronically complex polycyclic rings. Steric effects could prevent the coupling of the nucleophile through this technique; it is worth exploring the many potential solutions to this challenge.

It may be beneficial to use primary amines in the S_N2 reaction to increase the success of the coupling. The exposed N-H bonds can be protected using any one of a number of commonly used protection strategies, many of which are borrowed from peptide synthesis, to prevent the formation of a diazoamine products during the azo coupling. Protecting groups, such as TMS or TBS, would adequately protect the secondary amine without significantly deactivating the ring and preventing EAS from occurring.³⁷ Alternatively, secondary amines may coupled with the use of a stronger base, such as potassium *tert*-butoxide, or with additional heating if it is determined that the cellulose

membrane would maintain structural integrity under those conditions.³² Planar-solid phase synthesis is a technique that is primed for the use in the discovery of a novel colorimetric halide indicator through the synthesis of dye arrays. Once a structure activity relationship is determined for colorimetric anion indication, this technique can be applied to synthesize indicators for more hazardous anions, eventually allowing for easy detection of anions in the field or in regions where analytic instrumentation is out of reach.

Experimental methods

Microwave reactions were performed using a CEM Corp. (Matthews, NC) Discover LabMate system using the 10-mL or 35-mL reaction vessel. Thin-layer chromatography was performed using Merck KGaA TLC silica gel 60 W F254 plates. ¹H NMR spectra were acquired using a Varian Mercury Plus 400 spectrometer. Chemical shifts are reported in parts-per-million (ppm) and referenced according to the deuterated solvent for ¹H spectra (CH₃CN). A Cary 5000 UV-Vis NIR spectrophotometer equipped with an integrating sphere for diffuse reflectance measurements was utilized to analyze polystyrene resin- and cellulose-supported intermediates and products. Anhydrous solvents were purchased from Aldrich (pyridine, acetonitrile, dichloromethane, chloroform, *N*-methyl-2-pyrrolidone, *N,N*-dimethylformamide) and were used without further purification.

4-Hydroxyacetophenone (or alternative B ring) resin synthesis (6)

Merrifield resin **4**, 100 mg, 0.15 mmol/gram loading) was pre-swollen in a microwave vessel in dry NMP (0.5 mL) under N₂. 4-Hydroxyacetophenone (36 mg, 0.29 mmol) and cesium carbonated (90 mg, 0.27 mmol) were suspended in NMP (2.5 mL) and combined

with the pre-swelled resin in NMP. The mixture was heated by microwave irradiation to 150 °C for 5 min with stirring. Resin beads were transferred to a fritted glass filter and washed with acetone:methanol:water (1:1:1) (5 x 5 mL), acetone:methanol (1:1) (5 x 5 mL), acetone (5 x 5 mL), ethyl acetate (5 x 5 mL), CH₂Cl₂ (5 x 3 mL) and HPLC-grade pentane (5 x 5 mL) and dried under vacuum to yield 4-hydroxyacetophenone resin (**6**) as light brown resin beads. Products were stored in the refrigerator at 5 °C.

Aminal resin synthesis (**7**)

4-Hydroxyacetophenone resin (**6**, 100 mg) was pre-swelled in NMP (0.5 mL) and combined with naphthalene-1,8-diamine (1.59 g, 100 mmol) and catalytic methanesulfonic acid (MSA) (1 drop) in NMP (2.5 mL) and reacted at 80 °C for 30 min. Resin beads were transferred to a fritted glass filter and washed with acetone:methanol:water (1:1:1) (5 x 5 mL), acetone:methanol (1:1) (5 x 5 mL), acetone (5 x 5 mL), ethyl acetate (5 x 5 mL), CH₂Cl₂ (5 x 3 mL) and HPLC-grade pentane (5 x 5 mL) and dried under vacuum to yield aminal resin (**7**) as light brown resin beads. Products were stored in the refrigerator at 5 °C.

Brady's reagents (2, 4-dinitrophenol hydrazine) test for aldehydes and ketones²³

A 1 mg sample (or 1 cm x 1 cm for planar supports) of **10** was submerged in 3 mL of a 100 mM solution of 2, 4-dinitrophenol hydrazine (2 g, 10.1 mmol) in ethanol (100 mL, 95%) with H₂SO₄ (~4 drops). The suspension was warmed over a steam bath for 15 min; presence of an aldehyde or ketone was confirmed by the evolution of a bright orange color on the support.

Azo resin synthesis (**12**)

A mixture of 3-aminobenzoic acid (or other A-ring)(20.57 mg, 0.15 mmol), amination resin (or other B-ring) resin (**7**, 100 mg) and MSA (46.25 mg, 0.03 μ L, 0.48 mmol) in NMP (12 mL) was cooled to 0 °C. A solution of sodium nitrite (18 mg, 0.26 mmol) was dissolved in water (1.0 mL) and NMP (1.8 mL) and gradually added to the resin mixture over 10 min. The reaction was warmed to 20 °C and reacted for 40 min. The reaction was quenched with aqueous sodium bicarbonate (1 M, 5.0 mL) until neutral. Resin beads were transferred to a fritted glass filter and washed with acetone:methanol:water (1:1:1) (5 x 5 mL), acetone:methanol (1:1) (5 x 5 mL), acetone (5 x 5 mL), ethyl acetate (5 x 5 mL), CH₂Cl₂ (5 x 3 mL) and HPLC-grade pentane (5 x 5 mL) and dried under vacuum to yield 3-ABA-amination resin (**12**) as orange resin beads. Products were stored in the refrigerator at 5 °C.

Qualitative UV-vis colorimetric halide response analysis

Solutions (or suspensions) were prepared with 0.10 M methanesulfonic acid aqueous solution for absorbance values between 0.5-3 units. Additions of acidic halide (HBr, HCl) were made using a micropipette and a subsequent spectrum recorded for each addition. Following the acid halide additions, aqueous 1 M NaOH was added incrementally until the solution was basic with a spectrum recorded following each addition. After each individual halide test and subsequent pH test with base additions, the cuvette was clean and the procedure was repeated with each halide. Halide salt additions (KCl, KBr) were made to saturation and the resulting spectrum compared to the acidic halide additions.

Activation of Cellulose membrane (13)

The cellulose functionalization was adapted from a literature protocol.³² A 5 cm x 4.5 cm sheet of Whatman 1Chr paper (**13**) was immersed in a solution of trifluoroacetic acid (2.66 g, 0.02 mmol, 8.93 ml, 20%) in dichloromethane in a small pyrex dish for 10 min. The acid solution was then decanted and the sheet was washed with dichloromethane (2 x 10 mL) for 5 min each and then dried under a stream of N₂. The acid-swelled membrane was then immersed in a 2 M solution of tosyl chloride (38 g, 199 mmol, 8.93 mL) in pyridine and shaken for 24 hours. The TsCl solution was decanted and the sheet was washed with EtOH (2 x 10 mL) and DCM (1 x 10 mL) for 5 min each and dried under a stream of N₂, to yield tosyl chloride activated cellulose (**14**) as a white membrane.

Amination of activated cellulose membrane (15)

The tosyl chloride activated cellulose (**14**) was immersed in of 4,7,10-trioxatridecane-1,13-diamine (5.38 g, 24.4 mmol, 5.35 mL) and heated for 30 min in a 80 °C oven. The amine solution was then decanted and the paper washed with 10 mL portions of DMF, EtOH, 1.0 N NaOH (aq), H₂O, EtOH (2x) and DCM (5 min in each wash) and dried under a stream of N₂ to yield aminated cellulose (**15**) as a white membrane (186 $\mu\text{mol}/\text{cm}^2$), which was stored under an inert atmosphere.

Ninhydrin quantitation of amine loading protocol²³

A 0.5 cm x 0.5 cm piece of the membrane was cut off and immersed in a small test tube containing 1 mL of a 2 mM ninhydrin solution in ethanol. This solution was heated to 100 °C for 5 min to allow color to develop. The absorbance at 570 nm of the reaction solution was measured on the Cary 5000 UV-vis-NIR spectrophotometer and concentration was determined using its extinction coefficient.

Representative synthesis of α -chloro functionalized paper (**16**)

The amine support (**15**) was immersed in an ice-cold 1.5 M solution of α -chloroacetyl chloride (1.52 g, 13.5 mmol , 9.0 mL) in CH₂Cl₂ for 5 min. The solution was then decanted and an ice-cold 2 M solution of triethylamine (1.82 g, 18 mmol, 9.0 mL) in CH₂Cl₂ was added and allowed to react for 10 min. The solution was decanted and the paper was washed with portions of CH₂Cl₂ (10 mL) until the washing solution was clear. The paper was dried under a stream of N₂ to yield α -chloro cellulose (**16**) as an off-white membrane, which was stored under an inert atmosphere.

Student synthesis of Azo dye microarrays

Students in the second undergraduate organic chemistry class at The Ohio State University (Chem 2550, AU 2013) were placed in groups of two and given a 5 cm x 4.5 cm piece of α -chloro cellulose paper (**16**). Each class (10 groups) was given a stock solution (250 mL) of 25 mM B-ring (Figure 10) and saturated CsCO₃ in NMP. The students then placed their paper in a 35 mL microwave tube (two sheets per tube) and heated by microwave

irradiation to 150 °C for 5 min. The B-ring solution was decanted and the sheets were washed with DCM (2 x 25 mL) for 5 min each then dried under a stream of N₂. Each group then removed their sheet from the vessel and spotted a small aliquot of one of 14 choices of A-rings (Figure 11) in THF (saturated solution) in a grid according to the provided diagram (Figure 11). Once the spots were dried, the sheets were submerged in an aqueous solution of sodium nitrite (1 M) and methanesulfonic acid (0.2 M) until color developed on each spot (~10 min).

Synthesis of methyl orange cellulose membrane (**18**)

α -Chloro cellulose (**16**, 5 cm x 5 cm) was immersed in a 1 M solution of *N*-methylaniline (1.60 g, 15 mmol) with cesium carbonate 1.0 M (4.88 g, 15 mmol) in NMP (15 mL). The mixture was heated by microwave irradiation to 150 °C for 5 min with stirring. The reaction solution was decanted and the sheet was washed with DCM (2 x 25 mL) for 5 min each then dried under a stream of N₂ to yield *N*-methylaniline cellulose (**17**) as a white membrane.

4-Sulfanilic acid in THF (conc.) was spotted on to **17** (1 cm x 1 cm) and immersed in a concentrated aqueous solution of sodium nitrite (1 M) and methanesulfonic acid (0.2 M) until color developed. The reaction solution was decanted and the sheet was washed with DCM (2 x 25 mL) for 5 min each then dried under a stream of N₂ to yield methyl orange cellulose (**18**) as a white membrane with an orange-red spot.

Synthesis of 3-carboxybenzenediazonium tetrafluoroborate (**22**)

(General procedure for synthesis of aryldiazonium tetrafluoroborate salts)

3-Aminobenzoic acid (1.03 g, 7.5 mmol) was dissolved in tetrafluoroboric acid solution (1.1 mL, 48 wt%, 17.5 mmol) and cooled to 0 °C. A cold solution of sodium nitrite (1.7 g, 25 mmol) in water (3.5 mL) is added dropwise over 5 min. Once the addition is complete, the mixture is stirred for 10 min, and the product was isolated with a sintered glass filter. The solid diazonium tetrafluoroborate was washed with cold tetrafluoroboric acid (3.5 mL, 48 wt%), EtOH (2 x 3.5 mL, 190 proof), and diethyl ether (3 x 3.5 mL) to yield 3-carboxybenzenediazonium tetrafluoroborate (**22** 1.55 g, 6.5 mmol, 87.6 %) as a white powder. Product was used without further purification

Synthesis of (*E*)-3-((4-(methyl(phenyl)amino)phenyl)diazenyl)benzoic acid (**27**)(General procedure for azo coupling using aryldiazonium tetrafluoroborate salts)

N-Methyl-*N,N*-diphenylamine (55.3 mg, 0.302 mmol, 53 µL) was dissolved in acetonitrile (7.5 mL) at 0 °C. A suspension of **22** (141.9 mg, 0.604 mmol) and pyridine (1.2 mmol, 0.09 mL) in acetonitrile (7.5 mL) was added over 30 min. Reaction progress was checked by TLC for completion. Benzoic acid-type products can be isolated by water:diethyl ether extraction, followed by acidification and subsequent re-extraction by water:diethyl ether. (*E*)-3-((4-(methyl(phenyl)amino)phenyl)diazenyl)benzoic acid was isolated as an orange-red powder (**27**, 79.1 mg, 0.238 mmol, 78%); *R*_f (10% (v/v) isopropanol in hexanes) 0.11; ¹H NMR (400 MHz, CH₃CN) δ 3.41 (s, 3H), 6.91 (d, 2H), 7.26 (tt, 1H), 7.30 (dd, 2H), 7.46 (td, 2H), 7.63 (t, 1H), 7.83 (d, 2H), 8.05 (obs m, 2H), 8.37 (t, 1H).

Attempted synthesis of Wang- linker functionalized cellulose (**24**)

α -Chloro cellulose (**16**, 5 cm x 5 cm) was immersed in a 1 M solution of 4-hydroxybenzaldehyde (1.83 g, 15 mmol) with cesium carbonate 1.0 M (4.88 g, 15 mmol) in NMP (15 mL). The mixture was heated by microwave irradiation to 150 °C for 5 min with stirring. The reaction solution was decanted and the sheet was washed with DCM (2 x 25 mL) for 5 minutes each then dried under a stream of N₂ to yield 4-hydroxybenzaldehyde cellulose (**23**) as a white membrane.

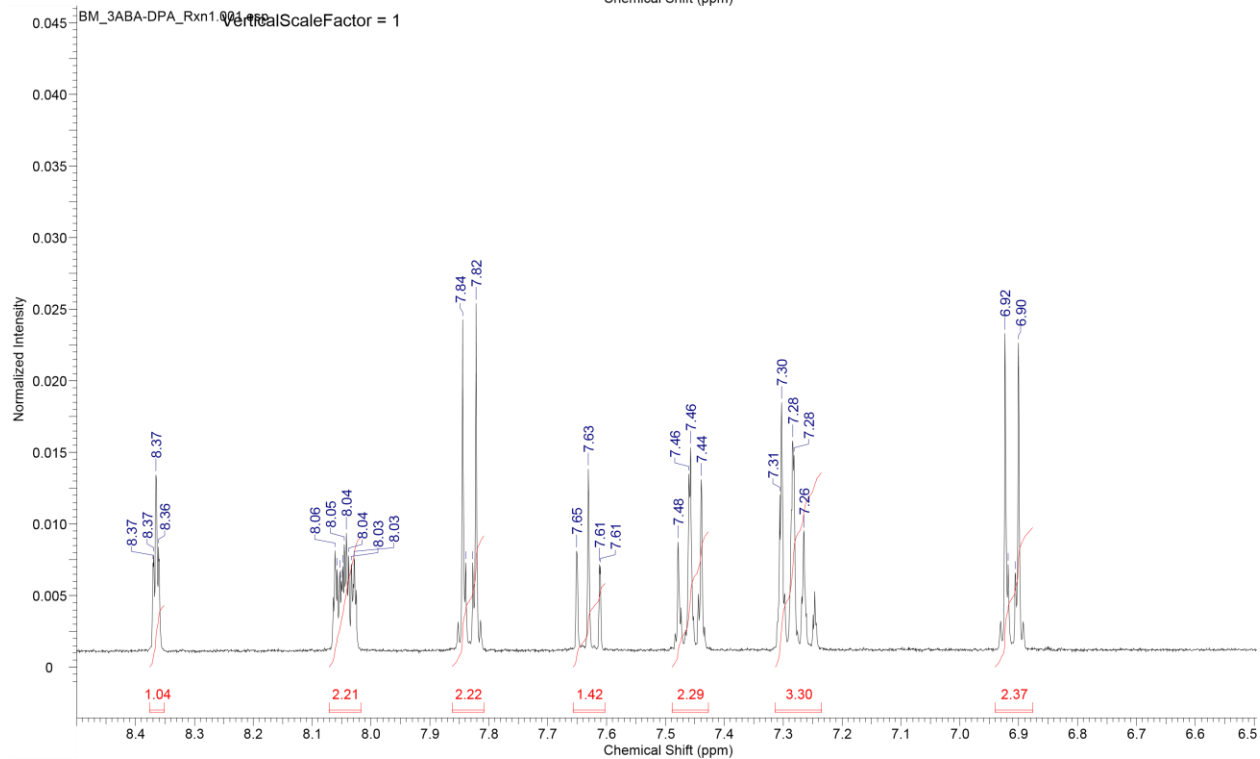
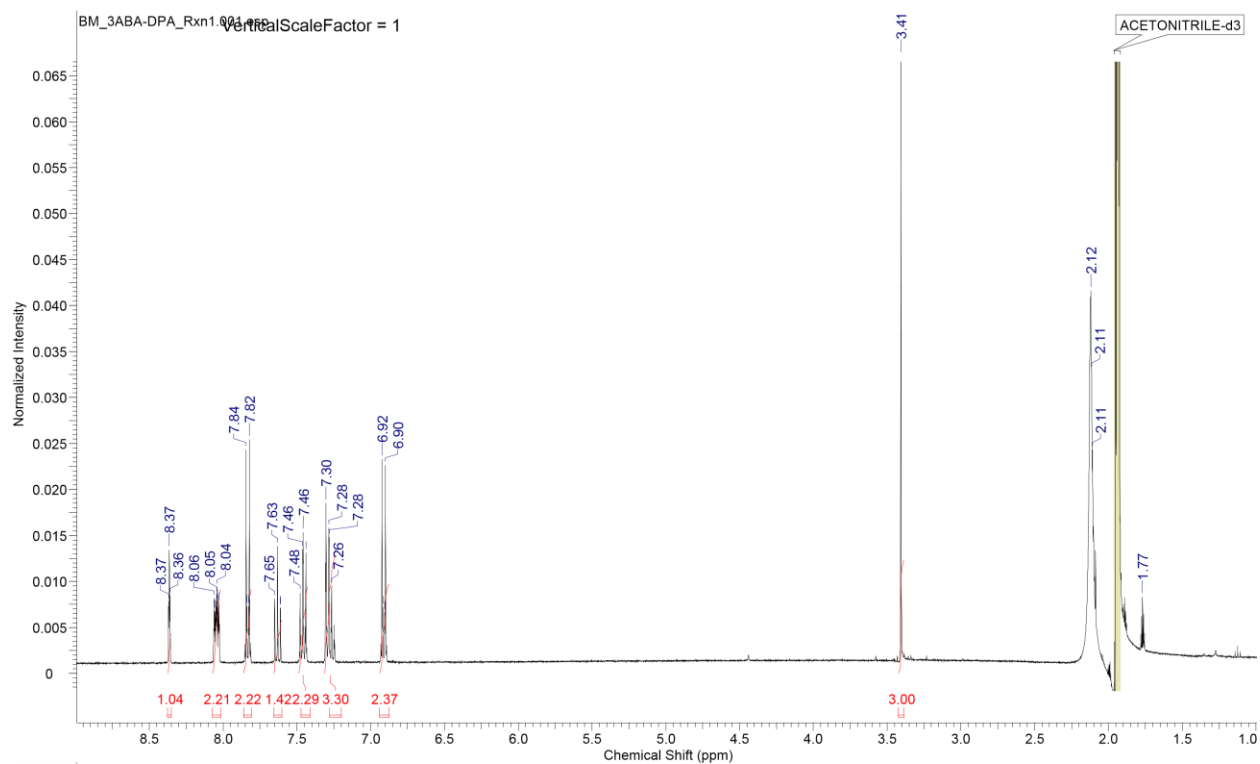
4-Hydroxybenzaldehyde cellulose (**23**) was immersed in a solution (30 mL) sodium borohydride (1 M, 1.13 g, 30 mmol) and sodium hydroxide (1 N, 1.20 g, 30 mmol) for 20 min with stirring at room temperature. The reaction solution was decanted and the membrane was washed with H₂O (2x), EtOH (2x), and CH₂Cl₂ (15 mL) for 5 min each before being dried under a stream of N₂ to yield 4-hydroxybenzyl alcohol cellulose (**24**) as a white membrane.

B-ring coupling of Wang-Functionalized cellulose

24 was immersed in tosyl chloride (1 M, 5.72 g, 30 mmol) in DMF (30 mL) for 1 H with shaking. This tosylation solution was decanted and membrane was washed with EtOH (2 x 10 mL) and DCM (1 x 10 mL) for 5 min each and dried under a stream of N₂. Resulting paper was submerged in diphenylamine (1 M, 5.07 g, 30 mmol, 4.23 mL) in DMF (30 mL) for at 80 °C for 30 min. Diphenylamine solution was decanted and membrane was washed with H₂O (2x), EtOH (2x), and CH₂Cl₂ (15 mL) for 5 min each and dried under a stream of N₂ to yield a white membrane as products were not formed

Appendix

1. ^1H NMR spectrum for **27** in CD_3CN



2. List of all Autumn 2013 REEL project supervising staff, organizers, and participants

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Faith Cahoon
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Sean Cody
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Special Thanks

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